Phytochemistry 69 (2008) 2716–2723

Contents lists available at ScienceDirect

Phytochemistry

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

Immunosuppressive pregnane glycosides from *Periploca sepium* and *Periploca forrestii*

Jiaquan Feng [a], Rujun Zhang [a], Yu Zhou [b], Zhenhua Chen [a], Wei Tang [b], Qunfang Liu [a], Jian-Ping Zuo [b,*], Weimin Zhao [a,*]

[a] Department of Natural Products Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China
[b] State Key Laboratory of New Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China

ARTICLE INFO

Article history:
Received 5 March 2008
Received in revised form 30 May 2008
Available online 1 October 2008

Keywords:
Periploca sepium
Periploca forrestii
Asclepiadaceae
Immunosuppressive
Pregnane glycoside

ABSTRACT

Nine pregnane glycosides containing peroxy functions in their sugar moieties (**1**–**5** and **11**–**14**), five oligosaccharides (**6**–**10**), six pregnane glycosides (**15**–**20**), and five cardiac glycosides (**21**–**25**) were isolated from the root barks of *Periploca sepium* Bge. (Asclepiadaceae) and the roots of *Periploca forrestii* Schltr. (Asclepiadaceae), two traditional Chinese medicines used for the treatment of rheumatoid arthritis. Among them, **1**–**8** are hitherto unknown. Their structures were characterized on the basis of spectroscopic analyses. In pharmacological testing, compounds **1**–**5** and **11**–**14** were found to exhibit inhibitory activity against the proliferation of T lymphocyte *in vitro* with IC_{50} values ranging from 0.29 μM to 1.97 μM, while the other components showed no significant inhibitory activity.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

The immunosuppressive drugs in current clinical use such as cyclosporin A, glycocorticoids, tacrolimus, and sirolimus, despite their undeniable clinical advantages, have rather serious side effects including liver toxicity, renal toxicity infection, malignancy, and other unwanted effects (Ader and Rostaing, 1998; Mignat, 1997; Wang et al., 2002; Wang, 2002; Smith et al., 2003). In the search for new potential immunosuppressive agents with high efficacy and low toxicity, we turned our attention to traditional Chinese medicines, which have been used in healthcare and disease treatment by the Chinese for thousands of years.

The genus *Periploca* belongs to Asclepiadaceae family and is widely distributed in north and tropical Africa, Orient and East Asia. The root barks of *Periploca sepium* Bge. (Asclepiadaceae) and the roots of *Periploca forrestii* Schltr. (Asclepiadaceae) have been used as traditional Chinese medicines for the treatment of rheumatoid arthritis and wounds; however, the toxicity in high-dose was often observed due to the existence of cardiac glycosides (Jiangsu New Medical College, 1998). Previous phytochemical studies on *P. sepium* resulted in the identification of pregnane glycosides, cardiac glycosides, oligosaccharides, coumarins, flavonoids and triterpenoids (Sakuma et al., 1969, 1971, 1980; Kasai et al., 1972; Kawanishi et al., 1972a,b, 1977; Ishizone et al., 1972; Oshima et al., 1987; Itokawa et al., 1987, 1988a–d; Komissarenko et al., 1983; Xu et al., 1990; Wang et al., 2007a,b; Ma et al., 2007), and the chemical investigation on *Periploca forrestii* mainly led to the isolation of some cardiac glycosides (Hu and Mu, 1989; Hu et al., 1990; Zhang et al., 2006a,b; Qiu et al., 2006); however the exact components with the activity against rheumatoid arthritis have not been explored. In our search for new immunosuppressive compounds from a series of traditional Chinese medicine reported with anti-rheumatoid arthritis effect, the crude extract of *Periploca sepium* was found to exhibit inhibitory activity against the proliferation of T cells, and periplocoside E was identified to inhibit the T cell proliferation and experimental allergic encephalomyelitis (Zhu et al., 2006a,b). Structure-activity relationship, however, was unknown due to the lack of a series of analogues. As our further study on this subject, systematic chemical investigation was undertaken on *P. sepium* and *P. forrestii*. We herein report the isolation and structure determination of nine pregnane glycosides containing peroxy functions in their sugar moieties (**1**–**5** and **11**–**14**), five oligosaccharides (**6**–**10**), six pregnane glycosides (**15**–**20**), and five cardiac glycosides (**21**–**25**). Among them, **1**–**8** are new compounds. Their structures were characterized on the basis of spectroscopic analyses. In the pharmacological test, nine pregnane glycosides containing peroxy function (**1**–**5** and **11**–**14**) were found to exhibit inhibitory activity against the proliferation of T lymphocyte *in vitro* with IC_{50} values ranging from 0.29 μM to 1.97 μM, while the other components showed no significant inhibitory activity.

* Corresponding authors. Tel.: +86 21 50806052; fax: +86 21 50806701.
E-mail addresses: jpzuo@mail.shcnc.ac.cn (J.-P. Zuo), wmzhao@mail.shcnc.ac.cn (W. Zhao).

0031-9422/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2008.08.015

2. Results and discussion

Compound **1** gave a $[M + Na]^+$ peak at *m/z* 1405.7112 in the HR–ESIMS, consistent with a molecular formula of $C_{70}H_{110}O_{27}$. Its ^{13}C NMR spectroscopic data established that the 3-*O*-glycosylated aglycone of **1** was identical to that of the known natural product periplocoside A (**11**) (Table 1). The ^1H NMR spectrum of **1** displayed five doublet anomeric proton signals at δ_H 4.35 (*d*, *J* = 8.0 Hz), 4.70 (br *d*, *J* = 9.5 Hz), 4.50 (br *d*, *J* = 9.0 Hz), 4.95 (br *d*, *J* = 9.6 Hz), and 4.55 (br *d*, *J* = 9.0 Hz), four methoxy resonances at δ_H 3.38 (*s*), 3.42 (*s*), 3.40 (*s*), 3.58 (*s*), and three singlet methyl signals at δ_H 2.05 (*s*), 0.70 (*s*), and 0.95 (*s*). The six sugar residues were identified as one β-cymaropyranose unit, one 2-*O*-acetyl-β-digitalopyranose unit, one β-digitoxopyranose unit, two β-canaropyranose units, and one 3, 7-dideoxyheptulose unit by analysis of the 2D-NMR and selective 1D-TOCSY spectra. Due to the selectivity of the multi-step coherence transfer, the 1D-TOCSY method allowed the subspectrum of a single monosaccharide unit to be extracted from the seriously overlapped region. In our experiments, selective 1D-TOCSY by irradiating each anomeric proton signal and each doublet methyl resonance yielded the subspectrum of each sugar residue from which the coupling constants of all protons in each sugar unit could be obtained. Combinational analyses of 1D-TOCSY, ^1H–^1H COSY, HMQC, and HMBC spectra allowed the full assignments of the proton and carbon resonances of each sugar. For instance, selective irradiation of the anomeric proton signal at δ_H 4.70 ppm gave an 1D-TOCSY spectrum containing proton resonances at δ_H 4.70 (br *d*, *J* = 9.5 Hz, H-1_{cym}), 2.09 and 1.55 (*m*, H-2_{cym}), and 3.78 (br *s*, H-3_{cym}), while selective irradiation of the doublet methyl signal at δ_H 1.17 ppm yielded the 1D-TOCSY spectrum containing proton resonances at δ_H 3.19 (*dd*, *J* = 9.4, 2.6 Hz, H-4_{cym}), 4.00 (*dq*, *J* = 9.4, 6.2 Hz, H-5_{cym}), and 1.17 (*d*, *J* = 6.2 Hz, H-6_{cym}). In the combinational analyses of its ^1H–^1H COSY spectrum, chemical shifts and also coupling constants of each proton of the sugar unit were determined (Table 2). The small coupling constants between H-3 and H-2, and between H-3 and H-4 indicated H-3 to be in equatorial orientation, while the relative large coupling constant (*J* = 9.4 Hz) between H-4 and H-5 establishing the axial orientation of H-4. The complete assignments of each carbon signal in the sugar moiety were made by analysing the HSQC spectrum of **1**. Further analysis of its HMBC spectrum demonstrated that the ^1H–^{13}C long-range correlation signal between the methoxyl proton resonance at δ_H 3.42 (*s*) and δ_C 76.2 (C-3_{cym}), which enabled the identification of the sugar unit as cymarose. The β-linkage of the cymarose was established by the large coupling constants (*J* = 9.5 Hz) of the anomeric proton signal. All other sugar units were also determined by using the above method. In addition, a similar sequence of protons from C-3 to C-7 due to 3, 7-dideoxyheptulose was noted, but no anomeric proton was observed in this heptulose unit. Further analyses of the HSQC, HMBC and TOCSY led to the establishment of the 3,7-dideoxy-4-methoxy-2-heptulose. The sequence of the six sugars in **1** was thus established as 2-*O*-acetyl-β-digitalopyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 4)-*O*-β-canaropyranosyl(1 → 4)-*O*-β-digitoxopyranosyl(1 → 5)-*O*-3, 7-dideoxy-4-methoxy-2-heptulopyranosyl(2 → 4)-dioxy-(1 → 3)-*O*-β-canaropyranosyl by the HMBC spectrum, in which ^1H–^{13}C long-range correlation signals were observed at H-1_{digta}/C-4_{cym}, H-1_{cym}/C-4_{canI}, H-1_{canI}/C-4_{digito}, H-1_{digito}/C-5_{hep}, H-1_{hep}/C-3_{canII}. The sugar chain was located at C-20 by the ^1H–^{13}C long-range correlation signal between the anomeric proton of canarose at δ_H 4.55 (br *d*, *J* = 9.0 Hz) and C-20 at δ_C 83.0. Besides, the characteristic resonance at δ_C 113.9 (C-2_{hep}) due to the peroxy bond between C-2_{hep} and C-4_{canII} was also observed (Itokawa et al., 1988a). The structure of this peroxy fragment was previously determined by chemical degradation by Itokawa's group (Itokawa et al., 1988b). Thus, the structure of **1** was characterized as the new pregn-5-ene-3 β,17α,20(*S*)-triol-3-*O*-(4,6-dideoxy-3-methoxy-2-hexosuloside-3-ene)-20-*O*-2-*O*-acetyl-β-digitalopyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 4)-*O*-β-canaropyranosyl(1 → 4)-*O*-β-digitoxopyranosyl(1 → 5)-*O*-3,7-dideoxy-4-methoxy-2-heptulopyranosyl(2 → 4)-dioxy-(1 → 3)-*O*-β-canaropyranoside, and assigned the trivial name periperoxide A.

Compound **2** was obtained as white amorphous powder with an elemental formula of $C_{71}H_{112}O_{26}$ determined by HR–ESIMS and NMR analyses. The ^{13}C NMR spectroscopic data of **2** indicated that its 3-*O*-glycosylated aglycone was identical to that of **1**. The ^1H NMR spectroscopic data of **2** displayed five doublet anomeric protons at δ_H 4.75 (br *d*, *J* = 9.6 Hz), 4.70 (br *d*, *J* = 9.6 Hz), 4.50 (br *d*, *J* = 9.2 Hz), 4.90 (br *d*, *J* = 9.5 Hz) and 4.55 (br *d*, *J* = 9.1 Hz), four methoxy signals at δ_H 3.42 (*s*), 3.40 (*s*), 3.40 (*s*) and 3.60 (*s*), three singlet methyl resonances at δ_H 2.05 (*s*), 0.70 (*s*) and 0.94 (*s*). The six sugar residues were identified as one 4-*O*-acetyl-β-cymaropyranose unit, two β-cymaropyranose unit, one 4-*O*-methyl-2-heptulopyranose unit, and two β-canaropyranose units by selective 1D-TOCSY and 2D-NMR analyses. The interlinkage manner of the six sugar units was determined to be 4-*O*-acetyl-β-cymaropyranosyl(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-canaropyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 5)-*O*-3,7-dideoxy-4-*O*-methyl-2-heptulopyranosyl(2 → 4)-dioxy-(1 → 3)-*O*-β-canaropyranosyl by the HMBC spectrum, in which ^1H–^{13}C long-range correlation signals were observed at H-1_{cymI}/C-4_{cymII}, H-1_{cymII}/C-4_{canI}, H-1_{canI}/C-4_{cymIII}, H-1_{cymIII}/C-5_{hep}, H-1_{hep}/C-3_{canII}. Thus, the structure of **2** was characterized as the new pregn-5-ene-3β, 17α, 20(*S*)-triol-3-*O*-(4,6-dideoxy-3-methoxy-2-hexosuloside-3-ene)-20-*O*-4-*O*-acetyl-β-cymaro-pyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 4)-*O*-β-canaropyranosyl(1 → 4)-*O*-β-cymaropyranosyl-(1 → 5)-*O*-3,7-dideoxy-4-methoxy-2-heptulopyranosyl (2 → 4)-dioxy-(1 → 3)-*O*-β-canaropyranoside, and assigned the trivial name periperoxide B.

Compound **3**, isolated as a white amorphous powder, gave a $[M + Na]^+$ peak at *m/z* 1361.7274 in the HR–ESIMS, consistent with the molecular formula $C_{69}H_{110}O_{25}$. The ^1H NMR and ^{13}C NMR spectra showed that **3** possessed an identical 3-*O*-glycosylated aglycone to those in **1** and **2**. Further analyses of ^1H–^1H COSY, HSQC, HMBC, and TOCSY spectra established that the terminal sugar unit 4-*O*-acetyl-β-cymaropyranosyl in **2** was replaced by the β-olean-

Table 1
^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data for the aglycone moiety of compounds **1**–**5** ($CDCl_3$)

No.	**1**–**3**		**4**, **5**	
	^1H NMR	^{13}C NMR	^1H NMR	^{13}C NMR
1	1.05/1.80, *m*	37.2 *t*	1.05/1.80, *m*	37.1 *t*
2	1.90/1.60, *m*	29.3 *t*	1.95/1.60, *m*	31.5 *t*
3	3.64, *m*	78.5 *d*	3.50, *m*	71.5 *d*
4	2.37/2.25, *m*	38.4 *t*	2.25, *m*	42.2 *t*
5		140.2 *s*		140.6 *s*
6	5.31, *br s*	121.9 *d*	5.32, *br s*	121.5 *d*
7	1.95, *m*	31.8 *t*	1.95, *m*	31.8 *t*
8	1.45, *m*	31.8 *d*	1.46, *m*	31.8 *d*
9	0.94, *m*	49.6 *d*	0.94, *m*	49.5 *d*
10		36.6 *s*		36.5 *s*
11	1.50, *m*	20.5 *t*	1.49, *m*	20.5 *t*
12	1.48/1.70, *m*	30.9 *t*	1.49/1.70, *m*	30.8 *t*
13		45.2 *s*		45.2 *s*
14	1.75, *m*	51.0 *d*	1.76, *m*	51.0 *d*
15	1.12, *m*	23.4 *t*	1.12, *m*	23.4 *t*
16	1.57/1.90, *m*	38.3 *t*	1.57/1.90, *m*	38.3 *t*
17		85.4 *s*		85.3 *s*
18	0.95, *s*	19.3 *q*	0.94, *s*	19.3 *q*
19	0.70, *s*	14.0 *q*	0.70, *s*	14.0 *q*
20	3.70, *m*	83.0 *d*	3.70, *m*	83.0 *d*
21	1.25, *d*	17.0 *q*	1.26, *d*	17.0 *q*

Table 2
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data for the sugar moiety of compounds **1**–**5** ($CDCl_3$) (*J* in Hz)

No.	**1**		**2**, **5**		**3**, **4**	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
	Digta		Cym I		Olean	
1	4.35, *d* (8.0)	102.4 *d*	4.75, *br d* (9.6)	99.7 *d*	4.50, *br d* (9.4)	101.4 *d*
2	5.05, *dd* (9.8, 8.0)	70.7 *d*	1.70/2.18, *m*	35.1 *t*	1.47/2.31, *m*	35.3 *t*
3	3.25, *dd* (9.8, 3.3)	81.4 *d*	3.74, *br s*	75.1 *d*	3.10, *m*	75.4 *d*
4	3.84, *d* (3.3)	67.8 *d*	3.74, *dq* (9.6, 3.3)	75.0 *d*	3.13, *t* (9.5)	80.5 *d*
5	3.55, *m*	70.6 *d*	3.94, *dq* (9.6, 6.2)	67.6 *d*	3.28, *dq* (9.5, 6.0)	71.6 *d*
6	1.33, *d* (6.4)	16.4 *q*	1.15, *d* (6.2)	18.0 *q*	1.31, *d* (6.0)	18.0 *q*
-OAc	2.05, *s*	169.4/21.0	2.05, *s*	170.3/21.0		
3-OCH_3	3.38, *s*	57.4 *q*	3.37, *s*	58.2 *q*	3.36, *s*	56.3 *q*
	Cym		Cym II		Cym I	
1	4.70, *br d* (9.5)	99.3 d	4.70, *br d* (9.6)	99.3 *d*	4.70, *br d* (9.4)	99.3 *d*
2	2.09/1.55, *m*	35.6 *t*	1.60/2.16, *m*	35.4 *t*	1.60/2.16, *m*	35.5 *t*
3	3.78, *br s*	76.2 *d*	3.76, *br s*	77.1 *d*	3.76, *br s*	77.0 *d*
4	3.19, *dd* (9.4, 2.6)	82.9 *d*	3.23, *dd* (9.4, 2.8)	82.2 *d*	3.23, *dd* (9.5, 3.0)	82.2 *d*
5	4.00, *dq* (9.4, 6.2)	68.8 *d*	3.95, *dq* (9.4, 6.2)	69.1 *d*	3.95, *dq* (9.5, 6.2)	69.0 *d*
6	1.17, *d* (6.2)	17.5 *q*	1.24, *d* (6.2)	17.8 *q*	1.24, *d* (6.2)	17.8 *q*
3-OCH_3	3.42, *s*		3.40, *s*		3.40, *s*	
	Can I		Can I		Can I	
1	4.50, *br d* (9.0)	100.3 *d*	4.50, *br d* (9.2)	101.5 *d*	4.50, *br d* (9.2)	101.4 *d*
2	1.55/2.20, *m*	38.3 *t*	1.60/2.25, *m*	38.5 *t*	1.60/2.25, *m*	38.5 *t*
3	3.55, *m*	69.3 *d*	3.53, *m*	69.5 *d*	3.53, *m*	69.5 *d*
4	2.90, *t* (9.5)	87.6 *d*	2.90, *t* (9.4)	88.0 *d*	2.90, *t* (9.4)	88.0 *d*
5	3.38, *dq* (9.5, 6.0)	70.3 *d*	3.28, *dq* (9.4, 6.2)	70.4 *d*	3.28, *dq* (9.4, 6.0)	70.4 *d*
6	1.20, *d* (6.0)	17.7 *q*	1.23, *d* (6.2)	17.8 *q*	1.24, *d* (6.0)	17.8 *q*
	Digto		Cym III		Cym II	
1	4.95, *br d* (9.6)	98.4 *d*	4.90, *br d* (9.5)	98.5 *d*	4.90, *br d* (9.5)	98.4 *d*
2	1.62/2.08, *m*	37.0 *t*	1.50/2.10, *m*	35.8 *t*	1.50/2.10, *m*	35.8 *t*
3	4.18, *br s*	66.7 *d*	3.80, *br s*	76.7 *d*	3.80, *br s*	76.7 *d*
4	3.18, *dd* (9.5, 3.0)	82.5 *d*	3.20, *dd* (9.4, 2.8)	82.6 *d*	3.20, *dd* (9.4, 3.0)	82.5 *d*
5	3.79, *dq* (9.5, 6.3)	68.2 *d*	3.86, *dq* (9.4, 6.2)	68.8 *d*	3.86, *dq* (9.4, 6.0)	68.7 *d*
6	1.20, *d* (6.3)	18.1 *q*	1.20, *d* (6.2)	18.1 *q*	1.20, *d* (6.0)	18.1 *q*
3-OCH_3			3.42, *s*	58.4 *q*	3.42, *s*	58.4 *q*
	Heptu		Heptu		Heptu	
1	5.10/4.72, *d* (7.6)	86.3 *t*	5.10/4.72, *d* (7.7)	86.4 *t*	5.10/4.72, *d* (7.7)	86.3 *t*
2		113.6 *s*		113.7 *s*		113.6 *s*
3	2.42/1.55, *m*	36.5 *t*	2.42/1.55, *m*	36.7 *t*	2.42/1.55, *m*	36.7 *t*
4	3.48, *m*	77.6 *d*	3.49, *m*	77.6 *d*	3.48, *m*	77.6 *d*
5	3.24, *t* (9.6)	82.7 *d*	3.24, *t* (9.3)	82.6 *d*	3.24, *t* (9.6)	82.5 *d*
6	3.55, *dq* (9.6, 6.2)	69.8 *d*	3.55, *dq* (9.3, 6.0)	69.9 *d*	3.55, *dq* (9.6, 6.2)	69.9 *d*
7	1.25, *d* (6.2)	18.1 *q*	1.25, *d* (6.0)	18.2 *q*	1.25, *d* (6.2)	18.2 *q*
4-OCH_3	3.40, *s*	57.5 *q*	3.40, *s*	57.6 *q*	3.40, *s*	57.5 *q*
	Can II		Can II		Can II	
1	4.55, *br d* (9.0)	100.8 *d*	4.55, *br d* (9.1)	100.8 *d*	4.55, *br d* (9.0)	100.8 *d*
2	1.60/2.18, *m*	36.8 *t*	1.65/2.20, *m*	36.9 *t*	1.65/2.20, *m*	36.9 *t*
3	3.48, *m*	78.2 *d*	3.49, *m*	78.3 *d*	3.48, *m*	78.2 *d*
4	3.30, *t* (9.5)	79.1 *d*	3.30, *t* (9.4)	79.2 *d*	3.30, *t* (9.5)	79.1 *d*
5	3.35, *dq* (9.5, 6.0)	69.7 *d*	3.35, *dq* (9.4, 6.2)	69.8 *d*	3.35, *dq* (9.5, 6.0)	69.7 *d*
6	1.25, *d* (6.0)	17.9 *q*	1.29, *d* (6.2)	18.0 *q*	1.29, *d* (6.0)	18.0 *q*
	Hexo		Hexo		Hexo	
1	5.00, *s*	97.2 *d*	5.02, *s*	97.3 *d*	5.02, *s*	97.2 *d*
2		185.8 s		185.8 *s*		185.8 *s*
3		147.7 s		147.8 *s*		147.8 *s*
4	5.72, *s*	118.4 d	5.75, *s*	118.4 d	5.75, *s*	118.4 *d*
5	4.68, *m*	68.8 d	4.68, *m*	68.8 d	4.68, *m*	68.8 *d*
6	1.49, *d* (6.9)	22.9 *q*	1.48, *d* (6.8)	23.0 *q*	1.49, *d* (6.9)	23.0 *q*
3-OCH_3	3.58, *s*	54.9 *q*	3.60, *s*	54.9 *q*	3.61, *s*	54.9 *q*

drosyl unit in **3**. The structure of **3**, named periperoxide C, was thus established as the new pregn-5-ene-3β,17α,20(*S*)-triol-3-*O*-(4,6-dideoxy-3-methoxy-2-hexosuloside-3-ene)-20-*O*-olea-ndropyranosyl(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-canaropyranosyl-(1 → 4)-*O*-β-cymaropyra-nosyl(1→5)-*O*-3,7-dideoxy-4-methoxy-2-heptulopyranosyl(2 → 4)-dioxy-(1 → 3)-*O*-β-canaropyra-noside (see Fig. 1).

The structure of **4** was similar to that of **3**, and the structure of **5** similar to that of **2**, respectively, except the absence of 3-*O*-4, 6-dideoxy-3-methoxy-2-hexosuloside-3-ene in both **4** and **5** by HR-ESIMS and extensive NMR spectroscopic analyses. The structure of **4**, named periperoxide D, was finally determined as the new pregn-5-ene-3β,17α,20(*S*)-triol-20-*O*-oleandropyranosyl(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-canaropyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 5)-*O*-3,7-dideoxy-4-methoxy-2-heptulopyranosyl(2 → 4)-dioxy-(1 → 3)-*O*-β-canaropyranoside; and the structure of **5**, named periperoxide E, was characterized as the new pregn-5-ene-3β,17α, 20(*S*)-triol-20-*O*-4-*O*-acetyl-β-cymaropyranosyl(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-canaropyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 5)-*O*-3,7-dideoxy-4-methoxy-2-heptulopyranosyl-(2 → 4)-dioxy-(1 → 3)-*O*-β-canaropyranoside (see Fig. 2).

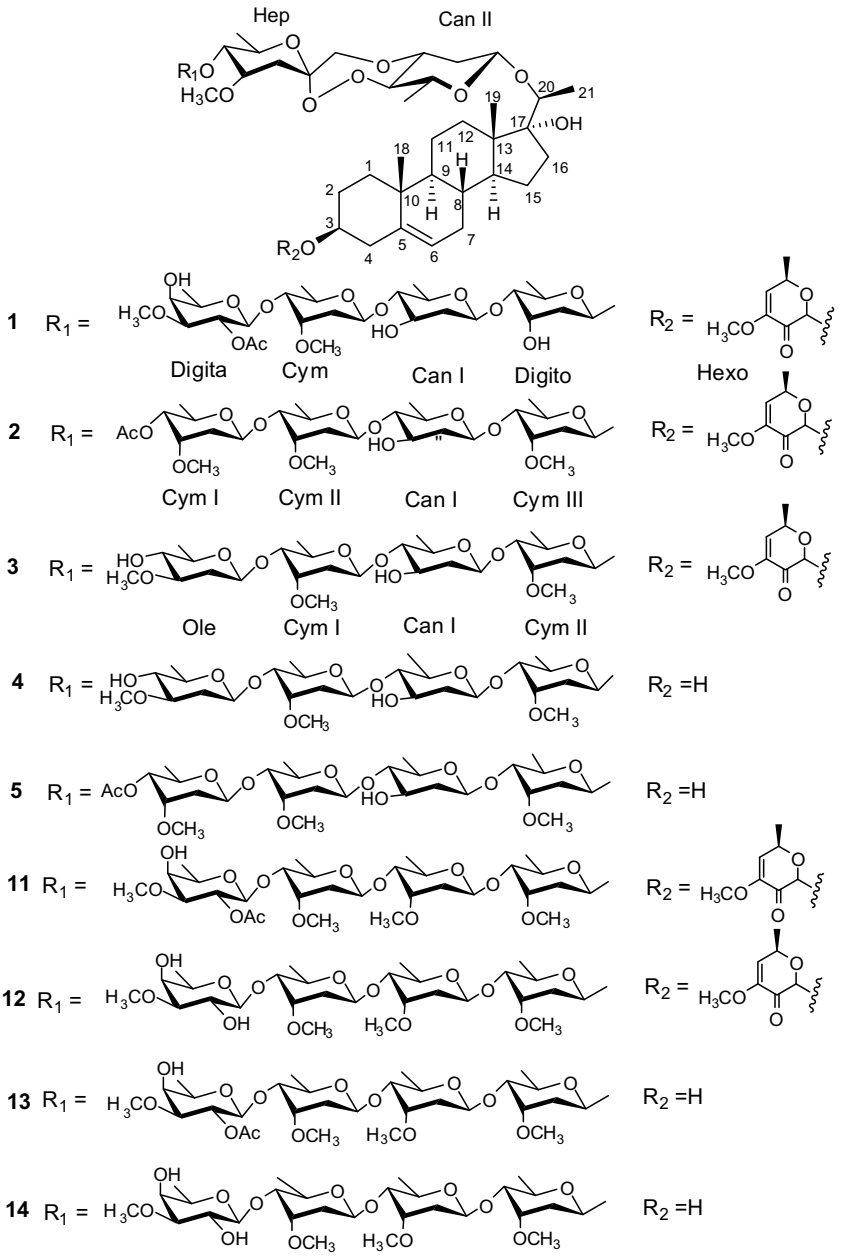

Fig. 1. Structures of compounds **1**–**5** and **11**–**14.**

Compound **6** was obtained as white amorphous powder with an elemental formula of $C_{36}H_{60}O_{18}$ as deduced from HR–ESIMS to NMR analyses. Its ^{1}H NMR exhibited four anomeric proton signals at δ_H 4.35 (*d*, *J* = 8.0 Hz), 4.70 (br *d*, *J* = 9.5 Hz), 4.50 (br *d*, *J* = 9.0 Hz), 4.87 (br *d*, *J* = 9.6 Hz) and four methoxy resonances at δ_H 3.37 (*s*), 3.44 (*s*), 3.43 (*s*), and 3.35 (*s*). The ^{13}C NMR spectrum of **6** showed 36 carbon signals separated by DEPT experiment into ten methyls, five methylenes, nineteen methines, and two quaternary carbons. The ^{1}H NMR and ^{13}C NMR spectra of **6** indicated it to be an oligosaccharide (Table 3). The five sugar units of **6** were identified as one 2-*O*-acetyl-β-digitalopyranose unit, one β-canaropyranose unit, one oleandronic acid-δ-lactone unit, and two β-cymaropyranose units by 1D-TOCSY and 2D-NMR analyses. The linkage of the five sugar moieties was established on the basis of its HMBC spectrum, in which, ^{1}H–^{13}C long-range correlation signals were found at H-1$_{dig}$/C-4$_{cymI}$, H-1$_{cymI}$/C-4$_{can}$, H-1$_{can}$/C-4$_{cymII}$, H-1$_{cymII}$/C-4$_{ole}$; and also on the basis of its NOESY spectrum, in which, NOE correlation resonances were observed between H-1$_{dig}$ and H-4$_{cymI}$,

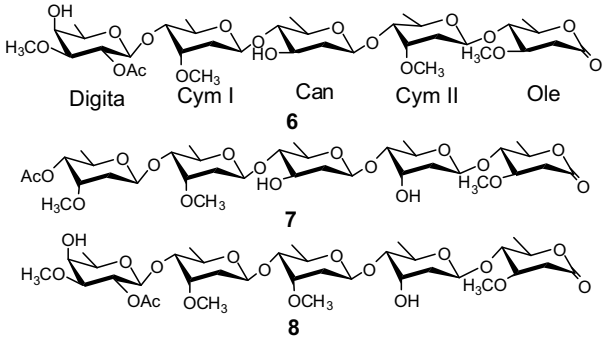

Fig. 2. Structures of compounds **6**–**8.**

Table 3
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectroscopic data of compounds **6–8** ($CDCl_3$) (*J* in Hz)

No.	**6**		**7**		**8**	
	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR
	Digita		Cym I		Digita	
1	4.35, *d* (8.0)	102.7 *d*	4.80, *br d* (9.6)	99.6 *d*	4.36, *d* (8.0)	102.7 *d*
2	5.08, *dd* (9.8, 8.0)	70.9 *d*	2.15/1.73, *m*	35.0 *t*	5.08, *dd* (9.6, 8.0)	71.0 *d*
3	3.25, *dd* (9.8, 3.3)	81.6 *d*	3.77, *br s*	75.0 *d*	3.25, *dd* (9.6, 3.2)	81.7 *d*
4	3.83, *d* (3.3)	68.0 *d*	4.50, *dd* (9.5, 3.0)	74.8 *d*	3.85, *d* (3.2)	68.1 *d*
5	3.55, *m*	70.6 *d*	3.95, *dq* (9.5, 6.3)	67.5 *d*	3.55, *m*	70.6 *d*
6	1.32, *d* (6.4)	16.7 *q*	1.16, *d* (6.3)	18.0 *q*	1.32, *d* (6.4)	16.8 *q*
3-OCH_3	3.37, *s*	57.5 *q*	3.39, *s*	58.2 *q*	3.39, *s*	57.6 *q*
-OAc	2.10, *s*	170.0/21.2	2.10, *s*	170.3/21.1	2.10, *s*	170.0/21.3
	Cym I		Cym II		Cym I	
1	4.70, *br d* (9.5)	99.4 *d*	4.72, *br d* (9.6)	99.3 *d*	4.72, *br d* (9.6)	99.9 *d*
2	2.10/1.55, *m*	35.7 *t*	2.15/1.60, *m*	35.2 *t*	2.10/1.55, *m*	36.1 *t*
3	3.80, *br s*	76.4 *d*	3.80, *br s*	76.7 *d*	3.78, *br s*	76.7 *d*
4	3.18, *dd* (9.4, 2.8)	83.0 *d*	3.24, *dd* (9.4, 2.8)	82.0 *d*	3.18, *dd* (9.5, 2.8)	83.8 *d*
5	3.92, *dq* (9.4, 6.2)	69.0 *d*	4.00, *dq* (9.4, 6.3)	69.1 *d*	3.89, *dq* (9.5, 6.3)	69.0 *d*
6	1.15, *d* (6.2)	17.7 *q*	1.14, *d* (6.3)	17.8 *q*	1.12, *d* (6.3)	18.2 *q*
3-OCH_3	3.44, *s*	58.7 *q*	3.44, *s*	58.4 *q*	3.43, *s*	58.9 *q*
	Can		Can		Cym II	
1	4.50, *br d* (9.0)	101.6 d	4.55, *br d* (9.0)	100.3 d	4.78, *br d* (9.5)	98.6 *d*
2	2.20/1.60, *m*	38.6 *t*	2.22/1.60, *m*	38.3 *t*	2.05 / 1.55, *m*	35.5 *t*
3	3.50, *m*	69.6 d	3.52, *m*	69.3 d	3.82, *br s*	77.0 *d*
4	2.90, *t* (9.5)	88.0 d	2.95, *t* (9.5)	87.7 d	3.15, *dd* (9.4, 2.8)	82.4 *d*
5	3.30, *dq* (9.5, 6.0)	70.6 d	3.30, *dq* (9.5, 6.0)	70.6 d	3.88, *dq* (9.4, 6.3)	68.3 *d*
6	1.22, *d* (6.0)	18.2 *q*	1.25, *d* (6.0)	17.8 *q*	1.19, *d* (6.3)	18.3 *q*
3-OCH_3					3.43, *s*	58.4 *q*
	CymII		Digito		Digito	
1	4.87, *br d* (9.6)	99.9 *d*	4.98, *br d* (9.6)	99.6 *d*	4.95, *br d* (9.6)	100.0 *d*
2	2.05/1.50, *m*	35.9 *t*	2.10/1.70, *m*	36.7 *t*	2.10/1.70, *m*	37.0 *t*
3	3.79, *br s*	77.0 *d*	4.22, *br s*	66.4 *d*	4.23, *br s*	66.4 *d*
4	3.11, *dd* (9.5, 3.0)	82.5 *d*	3.19, *dd* (9.5, 3.0)	82.3 *d*	3.15, *dd* (9.6, 3.2)	82.3 *d*
5	3.88, *dq* (9.5, 6.3)	68.9 *d*	3.81, *dq* (9.5, 6.3)	68.1 *d*	3.79, *dq* (9.6, 6.3)	68.5 *d*
6	1.20, *d* (6.3)	18.0 *q*	1.22, *d* (6.3)	18.0 *q*	1.21, *d* (6.3)	18.4 *q*
3-OCH_3	3.43, *s*	58.7 *q*				
	Ole		Ole		Ole	
1		170.8 s		170.4 s		170.8 s
2	2.70, *d* (3.6)	33.1 *t*	2.70, *d* (3.6)	32.9 *t*	2.72, *d* (3.6)	33.1 *t*
3	3.95, *m*	78.2 d	3.95, *m*	78.1 *d*	3.96, *m*	78.4 *d*
4	2.55, *t* (9.5)	80.8 d	2.54, *t* (9.5)	81.1 *d*	2.54, *t* (9.5)	81.4 *d*
5	4.14, *tq* (9.5, 6.5)	76.3 d	4.15, *tq* (9.5, 6.5)	76.0 *d*	4.15, *tq* (9.5, 6.5)	76.2 *d*
6	1.40, *d* (6.5)	19.2 *q*	1.40, *d* (6.5)	18.9 *q*	1.39, *d* (6.5)	19.2 *q*
3-OCH_3	3.35, *s*	57.0 *q*	3.35, *s*	56.8 *q*	3.36, *s*	57.0 *q*

H-1_{cymI} and H-4_{can}, H-1_{can} and H-4_{cymII}, and H-1_{cymII} and H-4_{ole}. Therefore, compound **6** was determined to be the new 2-*O*-acetyl-β-digitalopyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 4)-*O*-β-canaropyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 4)-*O*-oleandronic acid- δ-lactone, and assigned the trivial name perisac-charide A.

Compound **7,** obtained as a white amorphous powder, had the empirical molecular formula $C_{35}H_{58}O_{17}$ deduced from HR–ESIMS to NMR spectroscopic analyses. The ^{1}H NMR and ^{13}C NMR spectra indicated that the structure of **7** was similar to that of **6**. The five sugar units of **7** were identified as one 4-*O*-acetyl-β-cymaropyranose unit, one β-cymaropyranose unit, one β-canaropyranose unit, one β-digitoxopyranose unit, and one oleandronic acid-δ-lactone unit by 1D-TOCSY and 2D-NMR spectroscopic analyses. The structure of the sugar chain was established on the basis of its HMBC spectrum, in which, ^{1}H–^{13}C long-range correlation signals were observed at H-1_{cymI}/C-4_{cymII}, H-1_{cymII}/C-4_{can}, H-1_{can}/C-4_{dig}, and H-1_{dig}/C-4_{ole}; and also according to its NOESY spectrum, in which, NOE correlation resonances were found between H-1_{cymI} and H-4_{cymII}, H-1_{cymII} and H-4_{can}, H-1_{can} and H-4_{dig}, and H-1_{dig} and H-4_{ole}. Therefore, compound **7** was characterized as 4-*O*-acetyl-β-cymaropyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 4)-*O*-β-canaropyranosyl(1 → 4)-*O*-β-digitoxopyranosyl(1 → 4)-*O*-oleandronic acid-δ-lactone. It is a new compound and has been given the trivial name perisaccharide B.

Compound **8** was obtained as white amorphous powder with an elemental formula of $C_{36}H_{60}O_{18}$ deduced from HR–ESIMS and NMR analyses. The ^{1}H NMR and ^{13}C NMR spectra of **8** indicated it to be also an oligosaccharide. The five sugar units of **8** were identified as one 2-*O*-acetyl-β-digitalopyranose unit, one β-digitoxopyranose unit, one oleandronic acid-δ-lactone unit, and two β-cymaropyranose units by 1D-TOCSY and 2D-NMR analyses. The sequence of the sugar moieties was further established according to its HMBC and NOSEY spectra, and the structure of **8**, named perisaccharide C, was finally identified to be the new 2-*O*-acetyl-β-digitalopyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 4)-*O*-β-cymaropyranosyl(1 → 4)-*O*-β-digitoxopyranosyl(1 → 4)-*O*-oleandronic acid-δ-lactone.

In addition to the eight new compounds (**1**–**8**), 17 known compounds were also isolated and characterized by comparison with literature data as oligosaccharides D_2 (**9**), F_2 (**10**) (Kawanishi et al., 1977), periplocosides A (**11**) (Itokawa et al., 1988a), D (12) (Itokawa et al., 1988b), E (13) (Itokawa et al., 1988b), F (14) (Itokawa et al., 1988c), N (15) (Itokawa et al., 1988b) and M (16) (Itokawa et al., 1988b), periplocogenin (17) (Itokawa et al., 1987), pregn-ene-3β,20(*S*)-diol-3-O-[2-*O*-acetyl-β-D-digitalopyranosyl-(1 → 4)-*O*-β-D-cymaropyranoside]-20-*O*-β-D-glucopyranosyl(1 → 6)-*O*-β-D-glucopyranosyl(1 → 2)-*O*-β-D-digitalopyranoside (**18**) (Itokawa et al., 1988d), glycoside K (19) (Sakuma et al.,1971), and pregn-5-ene-3

Table 4
In vitro T cell proliferation inhibitory activity of Compounds **1**–**5** and **11**–**14**

Compound	CC_{50} (μM)	T cell proliferation inhibition	
		IC_{50} (μM)	SI[a]
Periperoxide A (**1**)	9.4	1.01	9.3
Periperoxide B (**2**)	48.2	1.32	36.5
Periperoxide C (**3**)	6.9	0.52	13.3
Periperoxide D (**4**)	15.7	0.82	19.1
Periperoxide E (**5**)	12.3	1.97	6.2
Periplocoside A (**11**)	18.7	0.51	36.7
Periplocoside D (**12**)	>10.0	0.29	>34.5
Periplocoside E (**13**)	10.1	0.64	15.8
Periplocoside F (**14**)	4.0	1.13	3.5

[a] Selectivity index [SI] is determined as the ratio of the concentration of the compound that reduced cell viability to 50% (CC_{50}) to the concentration of the compound needed to inhibit the proliferation to 50% (IC_{50}) of the control value. The immunosuppressant of rapamycin and ciclosporin A showed their inhibition activities on ConA-induced T cell proliferation, the IC_{50} value of rapamycin is 0.19 μM, ciclosporin A is 0.27 μM.

β,16 β,20(*R*)-triol-20-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl(1 → 2)-*O*-β-D-digitalopyranoside (**20**) (Itokawa et al., 1988d), periplocin (21) (Xu et al., 1990), periplocymarin (**22**) (Xu et al., 1990), periplofenin (**23**) (Xu et al., 1990), periforoside I (**24**) (Hu et al., 1990) and periforgenin A (**25**) (Hu et al., 1990). All the known compounds were isolated before from the same plant material.

All isolates were evaluated for inhibitory activity against proliferation of T lymphocyte *in vitro*. As a result, the nine peroxy function containing pregnane glycosides (**1**–**5** and **11**–**14**) showed significant activities against the proliferation of T lymphocyte *in vitro* without obvious cytotoxicity (Table 4), however, the five oligosaccharides (**6**–**10**) and the six pregnane glycosides (**15**–**20**) without peroxy function in their structures exhibited no such activity at up to 10 μM. These results suggested that compounds **1**–**5** and **11**–**14** may contribute in part to the therapeutic effect of *P. sepium* and *P. foresstii* against rheumatoid arthritis, and the peroxy function in the sugar chain of the pregnane glycosides is essential for the immunosuppressive activity. To our best knowledge, the famous antimalaria natural product artemisinin with peroxy function in its structure has been used to treat rheumatoid arthritis in China (Sun et al., 1991), and artemether, the derivative of artemisinin, has recently been demonstrated to inhibit T-cell proliferation and proliferation both *in vitro* and *in vivo* in our pharmacological investigation (Wang et al., 2007). Further investigation in the SAR of peroxy function and immunosuppressive effect seems warranted to the discovery of promising lead compound.

2.1. Concluding remarks

In summary, we have elucidated a series of active ingredients contributing to the therapeutic effect of *Periploca sepium* and *P. foresstii* against rheumatoid arthritis. Although these peroxy function containing pregnane glycosides have complicated structures compared to small molecular drugs, they could represent a type of efficient therapeutic agents against some refractory diseases, and separation of these compounds with those toxic cardiac glycosides existing in the *Periploca* plants may enable the development of safer immunosuppresive herbal product.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with Perkin–Elmer 241MC polarimeter, whereas IR spectra were recorded using a Perkin–Elmer 577 spectrometer. HR–ESIMS data were obtained on a Mariner spectrometer. NMR spectra were run on Bruker AM 400 spectrometer with TMS as internal standard. 1D-TOCSY spectra were run on a INOVA-600 spectrometer. Preparative HPLC was carried out using a Varian SD-1 instrument, equipped with a Merck NW25 C_{18} column (10 μM, 20 mm × 250 mm), and ProStar 320 UV/Vis Detector. Column chromatographic (CC) separations were carried out using silica gel H60 (300–400 mesh) and zcx-II (100–200 mesh) (Qingdao Haiyang Chemical Group Corporation, Qingdao, People's Republic of China) as packing materials. HSGF254 silica gel TLC plates (Yantai Chemical Industrial Institute, Yantai, People's Republic of China) and RP-18 WF_{254} TLC plates (Merck) were used for analytical TLC.

3.2. Plant material

Root bark of *P. sepium* used in this experiment was purchased from the Shanghai Huayu Herb Medicine Cooperation in 2001. The roots of *P. forrestii* were collected in Guizhou province, China in April, 2007. Both of the two plant materials were identified by Prof. Jingui Shen of Shanghai Institute of Materia Medica, and voucher specimens were deposited in the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences under the control numbers No. 20010605 and No.20070404, respectively.

3.3. Extraction and Isolation

The dried root barks of *Periploca sepium* (15.0 kg) were extracted with EtOH-H_2O (95:5, v/v) (30.0 L × 3) under conditions of reflux for 6h. The extract was concentrated to dryness in vacuo, suspended in H_2O (2.0 L), and then extracted with $CHCl_3$ (2.0 L × 3), and *n*-BuOH (2.0 L × 3), successively, yielding $CHCl_3$ (762.5 g) and *n*-BuOH (75.2 g) extracts, respectively. The $CHCl_3$ extract (762.5 g) was subjected to silica gel H60 CC eluted with a petroleum ether-acetone gradient (5:1, 2:1, 1:1, 0:1) to give fractions A (601.3 g), B (15.8 g), C (48.2 g), and D (3.6 g). Fraction C (48.2 g) was passed over a RP-18 column eluted with a MeOH–H_2O gradient (5:5 → 10:0) to give fractions C1–C4. Perisaccharide A (**6**) (20.3 mg), B (**7**) (16.5 mg), C (**8**) (30.6 mg), and oligosaccharides D_2 (**9**) (52.3 mg), F_2 (**10**) (35.6 mg) were isolated from fraction C2 (4.6 g) by preparative HPLC (ODS) eluted with a MeOH–H_2O gradient (3:7 → 8:2). Periplocosides M (**16**) (792.3 mg), N (**15**) (1.3 g), periplocogenin (**17**) (23.0 mg) were isolated from fraction C3 (5.3 g) by Sephadex LH-20 eluted with EtOH and preparative HPLC eluted with a MeOH–H_2O gradient (4:6 → 10:0). Fraction C4 (7.8 g) was subjected to preparative HPLC eluted with a MeOH–H_2O gradient (4:6 → 10:0) to afford periplocosides A (**11**) (2.0 g) and E (**13**) (1.5 g). Periperoxide A (**1**) (33.0 mg), periplocosides D (**12**) (40.1 mg), F (**14**) (13.0 mg), periplocymarin (130.5 mg) (**22**) and periplofenin (2.0 g) (**23**) were obtained from fraction D (3.6 g) by preparative HPLC (ODS) eluted with a MeOH–H_2O gradient (3:7 → 8:2). The *n*-butanol extract (75.2 g) was separated by a silica gel H60 column eluted with a $CHCl_3$–MeOH gradient (10:1, 5:1, 2:1, 1:1) to give pregn-5-ene-3 β,20(*S*)-diol-3-*O*-[2-*O*-acetyl-β-D-digitalopyranosyl(1 → 4)-*O*-β-D-cymaropyrano-side]-20-*O*-β-D-glucopyranosyl(1 → 6)-*O*-β-D-glucopyranosyl(1 → 2)-*O*-β-D-digitalopyranoside] (1.4 g) (**18**), glycoside K (32.4 mg) (**19**), and pregn-5-ene-3 β,16 β,20(*R*)-triol-20-*O*-β-D-glucopyranosyl-(1 → 6)-*O*-β-D-glucopyranosyl(1 → 2)-*O*-β-D-digitalopyranoside (15.8 mg) (**20**) and periplocin (25.0 mg) (**21**).

The roots of *Periploca forrestii* (10.0 kg) were percolated with EtOH (20.0 L × 3) at room temperature for two weeks. The extract was concentrated to dryness in vacuo, and suspended in H_2O (1.5 L), and then extracted with $CHCl_3$ (2.0 L × 3), and *n*-BuOH (2.0 L × 3), to yield $CHCl_3$ (306.5 g) and *n*-butanol (52.6 g) extracts,

respectively. The $CHCl_3$ extract (306.5 g) was subjected to a silica gel H60 CC using petroleum ether-acetone (20:1, 10:1, 5:1, 2:1, 1:1) as eluent to give fractions A (252.5 g), B (2.3 g), and C (12.5 g). Fraction B (2.3 g) was further purified by RP-18 CC to afford periforgenin A (526.2 mg) (**25**). Fraction C (12.5 g) was subjected to preparative HPLC (ODS) eluted with a MeOH–H_2O gradient (3:7 → 8:2) to afford periperoxide B (**2**) (14.1 mg), C (**3**) (29.4 mg), D (**4**) (35.1 mg), and E (**5**) (54.0 mg). Periforoside I (35.2 mg) (**24**) was isolated from the *n*-BuOH extract by RP-18 column, eluted with a MeOH:H_2O gradient (3:7 → 9:1).

3.4. Periperoxide A (*1*)

White amorphous powder; $[\alpha]_D^{24}$: -14 (*c* 0.2, $CHCl_3$); IR (KBr) ν_{max} 3489, 2935, 1745, 1716, 1637, 1454, 1375, 1317, 1240, 1157, 1093, 1058, 1004 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 1405.7112 $[M + Na]^+$ (calcd for $C_{70}H_{110}O_{27}Na$, 1405.7132).

3.5. Periperoxide B (*2*)

White amorphous powder; $[\alpha]_D^{24}$: -6 (*c* 0.19, $CHCl_3$); IR (KBr) ν_{max} 3448, 2935, 1735, 1639, 1454, 1375, 1240, 1163, 1059, 1005 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 1403.7330 $[M + Na]^+$ (calcd for $C_{71}H_{112}O_{26}Na$, 1403.7340).

3.6. Periperoxide C (*3*)

White amorphous powder; $[\alpha]_D^{24}$: -17 (*c* 0.21, $CHCl_3$); IR (KBr) ν_{max} 3453, 2935, 1716, 1639, 1454, 1378, 1097, 1000, 1058, 1002 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 1361.7274 $[M + Na]^+$ (calcd for $C_{69}H_{110}O_{25}Na$, 1361.7234).

3.7. Periperoxide D (*4*)

White amorphous powder; $[\alpha]_D^{24}$: -12 (*c* 0.33, $CHCl_3$); IR (KBr) ν_{max} 3448, 2929, 1745, 1456, 1376, 1313, 1157, 1099, 1058, 1002 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 1221.6725 $[M + Na]^+$ (calcd for $C_{62}H_{102}O_{22}Na$, 1221.6760).

3.8. Periperoxide E (*5*)

White amorphous powder; $[\alpha]_D^{24}$: +5 (*c* 0.12, $CHCl_3$); IR (KBr) ν_{max} 3446, 2933, 1735, 1452, 1375, 1238, 1161, 1095, 1058, 1004 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Tables 1 and 2; HRESIMS *m*/*z* 1263.6840 $[M + Na]^+$ (calcd for $C_{64}H_{104}O_{23}Na$, 1263.6866).

3.9. Perisaccharide A (*6*)

White amorphous powder; $[\alpha]_D^{24}$: +28 (*c* 0.97, $CHCl_3$); IR (KBr) ν_{max} 3390, 2931, 1732, 1735, 1367, 1246, 1163, 1091, 1068, 1003, 879 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 3, HRESIMS *m*/*z* 803.3675 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_{18}Na$, 803.3677).

3.10. Perisaccharide B (*7*)

White amorphous powder; $[\alpha]_D^{24}$: +51 (*c* 0.26, $CHCl_3$); IR (KBr) ν_{max} 3442, 2975, 2935, 2902, 1754, 1736, 1405, 1371, 1243, 1167, 1056, 1005, 868, 729 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 3; HRESIMS *m*/*z* 773.3572 $[M + Na]^+$ (calcd for $C_{35}H_{58}O_{17}Na$, 773.3572).

3.11. Perisaccharide C (*8*)

White amorphous powder; $[\alpha]_D^{24}$: +58 (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3496, 2974, 2935, 2885, 1741, 1452, 1371, 1249, 1164, 1091, 1005, 868, 723 cm^{-1}; for ^1H and ^{13}C NMR spectroscopic data, see Table 3; HRESIMS *m*/*z* 803.3676 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_{18}Na$, 803.3677).

3.12. Preparation of spleen cells from mice

BALB/C mice were sacrificed and spleens were removed aseptically. A single cell suspension was prepared after cell debris and clumps were removed. Erythrocytes were lysed using ammonium chloride buffer solution. The isolated Lymphocytes were washed 3 times with PBS containing 2% FBS, and were resuspended in RPMI 1640 medium at the indicated concentration.

3.13. Cytotoxicity assay

Fresh spleen cells (5×10^5) were cultured in 96-well flat plates with 200 μL of RPMI 1640 media containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin in a humidified, 37 °C, 5% CO_2-containing incubator for 48 h, in the presence or absence of various concentrations of compounds **1**–**25**. 18 μL of MTT (5 mg/mL) was added to each well at the final 5 h culture. Then 90 μL of lysis buffer (10% SDS, 50% DMF, pH 7.2) was added to each well for 6–7 h and the absorbance values at 570 nm were read by microplate reader (Bio-Rad, Model 550) (Zhu et al., 2006a).

3.14. T cell function assay

5×10^5 of fresh spleen cells were cultured for 48 h at the same conditions as mentioned above. The cultures were stimulated with 5 μg/mL of concanavalin A (ConA) to induce T cells proliferative responses. Compounds **1**–**25** were added to cultures with indicated concentrations to test their bioactivities. Proliferation was assessed in terms of uptake of $[^3H]$-thymidine during last 8 h culture pulsing with 25 μCi of $[^3H]$-thymidine for each well, and then cells were harvested onto glass fiber filters by a Basic 96 harvester. The incorporated radioactivity was counted by a liquid scintillation counter (1540 MicroBeta Trilux, Perkin–Elmer Life Sciences) (Zhu et al., 2006a).

Acknowledgements

The research work was supported by National Natural Science Foundation of China (20672124), Science and Technology Commission of Shanghai Municipality (06DZ19721), and New Drug Discovery Research Program of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences (06G604J026).

References

Ader, J.L., Rostaing, L., 1998. Cyclosporin nephrotoxicity: pathophysiology and comparison with FK-506. Curr. Opin. Nephrol. Hypertens. 7, 539–545.
Hu, Y.J., Mu, Q.Z., 1989. The chemical constituents of *Periploca forrestii*. Acta Botanica Yunnanica 11, 465–470.
Hu, Y.J., Mu, Q.Z., Zheng, Q.T., He, C.H., 1990. New cardenolides of *Periploca forrestii*. Acta Chim. Sinica 48, 714–719.
Ishizone, H., Sakuma, S., Kawanishi, S., Shoji, J., 1972. Constituents of Chinese crude drug. Wujiapi. VII. Structure of glycoside E of Bei-Wujiapi. Chem. Pharm. Bull. 20, 2402–2406.
Itokawa, H., Xu, J.P., Takeya, K., 1987. Studies on chemical constituents of antitumor fraction from *Periploca sepium* Bge I. Chem. Pharm. Bull. 35, 4524–4529.
Itokawa, H., Xu, J.P., Takeya, K., Watanabe, K., Shoji, J., 1988a. Studies on chemical constituents of antitumor fraction from *Periploca sepium*. II. Structures of new pregnane glycosides, periplocosides A, B and C. Chem. Pharm. Bull. 36, 982–987.
Itokawa, H., Xu, J.P., Takeya, K., 1988b. Studies on chemical constituents of antitumor fraction from *Periploca sepium*. IV. Structures of new pregnane glycosides, periplocosides D, E, L and M. Chem. Pharm. Bull. 36, 2084–2089.

Itokawa, H., Xu, J.P., Takeya, K., 1988c. Studies on chemical constituents of antitumor fraction from *Periploca sepium*. V. Structures of new pregnane glycosides, periplocosides J, K, F and O. Chem. Pharm. Bull. 36, 4441–4446.

Itokawa, H., Xu, J.P., Takeya, K., 1988d. Pregnane glycosides from an antitumor fraction of *Periploca sepium*. Phytochemistry 27, 1173–1179.

Jiangsu New Medical College, 1998. Dictionary of Chinese Herb Medicines, Shanghai Scientific and Technologic Press: Shanghai, pp. 1683, 2388.

Kasai, R., Sachiko, S., Kawanishi, S., Shoji, J., 1972. Constituents of Chinese crude drug "wujiapi". VI. Studies on the aglycones of steroidal glycosides of Bei-wujiapi (2). Chem. Pharm. Bull. 20, 1869–1873.

Kawanishi, S., Sakuma, S., Shoji, J., 1972a. Constituents of the Chinese crude drug Wujiapi. V. Structure of glycoside H1 of Bei-Wujiapi. Chem. Pharm. Bull. 20, 469–475.

Kawanishi, S., Sakuma, S., Okino, H., Shoji, J., 1972b. Constituents of the Chinese crude drug Wujiapi. IV. Structure of a new acetylbiose from steroidal glycosides of Bei-Wujiapi. Chem. Pharm. Bull. 20, 93–96.

Kawanishi, S., Kasai, R., Sakuma, S., Shoji, J., 1977. Constituents of Chinese crude drug "wujiapi" VIII. On the structures of new oligosaccharides C1, D2, F1 and F2 of Bei-wujiapi. Chem. Pharm. Bull. 25, 2055–2060.

Komissarenko, N.F., Khvorost, P.P., Ivanov, V.D., 1983. Coumarins and cardenolides from *Periploca sepium*. Khimiya Prirodnykh Soedinenii 1, 102–103.

Ma, Y.M., Shi, Q.H., Kong, Y., 2007. Chemical constituents of stem bark from *Periploca sepium*. Chem. Nat. Compd. 43, 497–498.

Mignat, C., 1997. Clinically significant drug interactions with new immunosuppressive agents. Drug. Saf. 16, 267–278.

Oshima, Y., Hirota, T., Hikino, H., 1987. Periplosides A, B and C, steroidal glycosides of *Periploca sepium* root barks. Heterocycles 26, 2093–2098.

Qiu, M.H., Wang, J., Du, Jiang; Qiu, Shengxiang; Zhou, Lin., 2006. Antitumor compositions containing Periploca extracts of periforoside I and periplocin and others. Chinese Patent CN200510010837. 5, 11.

Sakuma, S., Ishizone, H., Kasai, R., Kawanishi, S., Shoji, J., 1969. On the structure of glycoside G and K of Bei-Wujiapi. Chem. Pharm. Bull. 17, 2183–2185.

Sakuma, S., Ishizone, H., Kasai, R., Kawanishi, S., Shoji, J., 1971. Constituents of Chinese crude drug "wujiapi". III. On the structure of glycoside G and K of Bei-wujiapi. Chem. Pharm. Bull. 19, 52–59.

Sakuma, S., Kawanishi, S., Shoji, J., 1980. Constituents of the Chinese crude drug "wujiapi". IX. Structure of glycoside H2, a potentiator of NGF-mediated nerve fiber outgrowth. Chem. Pharm. Bull. 28, 163–168.

Smith, J.M., Nemeth, T.L., McDonald, R.A., 2003. Current immunosuppressive agents: efficacy, side effects, and utilization. Pediatr. Clin. North Am. 50, 1283–1300.

Sun, X.Z., Xie, S.S., Long, Z.Z., 1991. Studies on the immunosuppressive effects of artemisinin and derivatives. Chin. J. Integr. Med. 11, 37–38.

Wang, J.X., Tang, W., Shi, L.P., Wan, J., Zhou, R., Ni, J., Fu, Y.F., Yang, Y.F., Li, Y., Zuo, J.P., 2007. Investigation of the immunosuppressive activity of artemether on T-cell proliferation and proliferation. Br. J. Pharmacol. 150, 652–661.

Wang, L., Yin, Z.Q., Zhang, L.H., Ye, W.C., Zhang, X.Q., Shen, W.B., Zhao, S.X., 2007a. Chemical constituents from root barks of *Periploca sepium*. Chin. Pharm. J. 32, 1300–1302.

Wang, L., Yin, Z.Q., Shen, W.B., Zhang, Q.W., Ye, W.C., 2007b. A new pregnane and a new diphenylmethane from the root barks of *Periploca sepium*. Helv. Chim. Acta 90, 1581–1585.

Wang, X.L., Tang, F., Li, H.M., 2002. Bad responses of common immunosuppressants in organ transplantation. J. Chin. Phys. 4, 1291–1293.

Wang, Y.R., 2002. Recent advances in immunosuppressive drugs. Chin. J. New Drugs 11, 512–515.

Xu, J.P., Takeya, K., Itokawa, H., 1990. Pregnanes and cardenolides from *Periploca sepium*. Phytochemistry 29, 344–346.

Zhang, Y.H., Chen, D.L., Wang, F.P., 2006a. Two novel cardiac glycosides from *Periploca forrestii*. Chin. J. Org. Chem. 26, 329–332.

Zhang, Y.H., Yang, Y.T., Chen, D.L., Wang, F.P., 2006b. Chemical constituents of *Periploca forrestii*. Chin. Trad. Herbal Drugs 37, 345–347.

Zhu, Y.N., Zhao, W.M., Yang, Y.F., Zuo, J.P., 2006a. Periplocoside E, an effective compound from *Periploca sepium* Bge, inhibited T cell proliferation in vitro and in vivo. J. Pharmacol. Exp. Ther. 316, 662–669.

Zhu, Y.N., Zhong, X.G., Feng, J.Q., Zhao, W.M., Zuo, J.P., 2006b. Periplocoside E Inhibits Experimental Allergic Encephalomyelitis by suppressing IL-12-dependent CCR5 expression and IFN-γ-dependent CXCR3 expression in T Lymphocytes. J. Pharmacol. Exp. Ther. 318, 1153–1162.