Five New Pregnane Glycosides from the Root Barks of *Periploca sepium*

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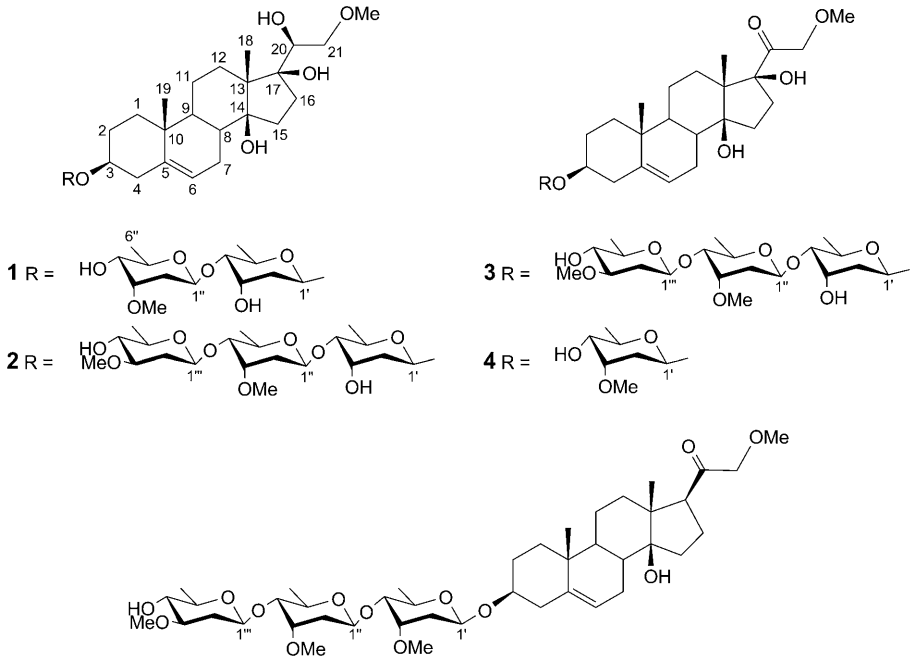
Five new pregnane glycosides, perisepiumosides A–E (**1**–**5**, resp.), along with the seven known constituents **6**–**12**, were isolated from the root barks of *Periploca sepium* BGE. (Asclepiadaceae), a traditional Chinese medicine used for the treatment of rheumatoid arthritis and wounds. Their structures were characterized on the basis of spectroscopic analyses.

Introduction. – The root barks of *Periploca sepium* BGE. (Asclepiadaceae) are used as Chinese traditional medicine for the treatment of rheumatoid arthritis and wounds [1]. Previous phytochemical studies on the *Periploca* species have led to the isolation of pregnane glycosides [2–9], cardiac glycosides [10], and oligosaccharides [11]. As part of our ongoing search for bioactive components from *Periploca sepium* [12][13], five new pregnane glycosides, perisepiumosides A–E (**1**–**5**), were isolated from the root barks of *Periploca sepium* along with seven known compounds **6**–**12**. We report herein the isolation and structural elucidation of these compounds.

Results and Discussion. – Compound **1** was obtained as a white amorphous powder with a molecular formula of $C_{35}H_{58}O_{11}$ determined by HR-ESI-MS (quasimolecular-ion peak at *m*/*z* 677.3891, $[M+Na]^+$), and by NMR analysis. The ^{1}H- and ^{13}C-NMR, ^{1}H,^{1}H-COSY, HMBC, and ROESY spectra allowed us to unambiguously assign all resonances of the aglycone of **1**. The two sugar units were identified as one digitoxose and one cymarose by selective 1D-TOCSY. Further analyses of HMBC and ROESY spectra confirmed its structure to be (3*β*,14*β*,17*β*,20*S*)-3,14,17,20-tetrahydroxy-21-methoxy-pregn-5-ene-3-*O*-*β*-cymaropyranosyl-(1 → 4)-*O*-*β*-digitoxopyranoside (**1**), a new compound, and was assigned the trivial name perisepiumoside A.

The ^{13}C-NMR spectrum of **1** showed 35 C-atom signals assigned by DEPT experiments into six Me, eleven CH_2, 13 CH, and five quaternary C-atoms (*Table 1*). The ^{1}H-NMR spectrum of **1** revealed the presence of one olefinic H-atom signal at *δ*(H) 5.39 (br. *s*), two MeO groups at *δ*(H) 3.43 (*s*) and 3.38 (*s*), and two *singlet* Me groups at *δ*(H) 1.19 (*s*) and 0.99 (*s*) (*Table 2*). Analyses of the ^{1}H,^{1}H-COSY and HMQC of **1** led to the deduction of fragments: C(1)–C(2)–C(3)–C(4), C(6)–C(7)–C(8)–C(9)–C(11)–C(12), C(15)–C(16), and C(20)–C(21) in the aglycone part. The planar structure of the aglycone was established on the basis of the HMBC spectrum, in which ^{1}H,^{13}C long-range correlation signals were observed between H–C(4)/C(6), C(10); H–C(6)/C(8), C(10); H–C(8)/C(15); H–C(9)/C(14);



H–C(12)/C(14); H–C(18)/C(12), C(13), C(14), C(17); H–C(19)/C(1), C(5), C(9), C(10); H–C(20)/C(16), C(17); and H–C(21)/C(22). The relative configuration of the aglycone of **1** was deduced from its ROESY spectrum as shown in the *Figure*. In particular, NOE correlation signals between H_α–C(20) and H_α–C(12) and H_β–C(12) instead of between H–C(21) and CH_2(12) revealed the configuration of C(20) to be (*S*) [14]. Accordingly, the aglycone of **1** was identified to be (3*β*,14*β*,17*β*,20*S*)-3,14,17,20-tetrahydroxy-21-methyoxypregn-5-ene [9]. In addition to the aglycone moiety, the ^{1}H-NMR spectrum of **1** exhibited two anomeric H-atom signals at δ(H) 4.70 (*dd*, $J = 9.6, 1.6$, 1 H) and 4.90 (*dd*, $J = 9.6, 1.6$, 1 H), and two Me *doublet*s at δ(H) 1.24 (*d*, $J = 6.4$) and 1.21 (*d*, $J = 6.2$) due to the two sugar units. Selective 1D-TOCSY by irradiating each anomeric H-atom signal and *doublet* Me signal yielded the four subspectra of the two sugar residues [15]. Selective irradiation of the anomeric H-atom signal at δ(H) 4.90 ppm gave a 1D-TOCSY spectrum containing the H-atom signals H–C(1′) at δ(H) 4.90 (*dd*, $J = 9.6, 1.6$), CH_2(2′) at δ(H) 2.07–2.13 (*m*) and 1.68–1.72 (*m*), and H–C(3′) at δ(H) 4.22 (br. *s*), while selective irradiation of the *doublet* Me signal at δ(H) 1.21 ppm yielded the 1D-TOCSY spectrum containing the H-atom signals H–C(4′) at δ(H) 3.20 (br. *d*, $J = 9.4$), H–C(5′) at δ(H) 3.78 (*dq*, $J = 9.4, 6.2$), and H–C(6′) at δ(H) 1.21 (*d*, $J = 6.2$). In combination with the ^{1}H,^{1}H-COSY spectrum, chemical shifts and also coupling constants of each H-atom of the sugar unit were determined (*Table 2*). The small coupling constants between H–C(3′) and H–C(2′), and between H–C(3′) and H–C(4′) indicated H–C(3′) to be in equatorial orientation, while the relatively large coupling constant ($J = 9.4$ Hz) between H–C(4′) and

Table 1. *^{13}C-NMR Data of* **1**–**5** (100 MHz, in $CDCl_3$)

	1	**2**	**3**	**4**	**5**
1	37.2 (*t*)	37.2 (*t*)	37.0 (*t*)	37.1 (*t*)	37.2 (*t*)
2	29.4 (*t*)	29.4 (*t*)	29.4 (*t*)	29.4 (*t*)	29.5 (*t*)
3	77.6 (*d*)	77.5 (*d*)	77.3 (*d*)	77.3 (*d*)	77.5 (*d*)
4	38.6 (*t*)	38.5 (*t*)	38.6 (*t*)	38.6 (*t*)	38.6 (*t*)
5	139.3 (*s*)	139.3 (*s*)	140.0 (*s*)	140.1 (*s*)	139.0 (*s*)
6	121.9 (*d*)	121.8 (*d*)	120.7 (*d*)	120.8 (*d*)	122.1 (*d*)
7	26.1 (*t*)	26.0 (*t*)	25.9 (*t*)	25.9 (*t*)	27.3 (*t*)
8	36.2 (*d*)	36.2 (*d*)	37.8 (*d*)	37.9 (*d*)	36.1 (*d*)
9	46.0 (*d*)	46.0 (*d*)	45.9 (*d*)	45.9 (*d*)	45.8 (*d*)
10	36.8 (*s*)	36.8 (*s*)	36.8 (*s*)	36.9 (*s*)	37.0 (*s*)
11	20.2 (*t*)	20.2 (*t*)	20.2 (*t*)	20.3 (*t*)	20.7 (*t*)
12	32.1 (*t*)	32.0 (*t*)	31.8 (*t*)	31.9 (*t*)	38.4 (*t*)
13	50.6 (*s*)	50.6 (*s*)	51.4 (*s*)	51.5 (*s*)	49.3 (*s*)
14	86.7 (*s*)	86.7 (*s*)	89.1 (*s*)	89.2 (*s*)	85.1 (*s*)
15	31.6 (*t*)	31.6 (*t*)	31.4 (*t*)	31.5 (*t*)	34.4 (*t*)
16	31.9 (*t*)	31.9 (*t*)	33.5 (*t*)	33.6 (*t*)	24.4 (*t*)
17	87.8 (*s*)	87.8 (*s*)	93.2 (*s*)	93.2 (*s*)	57.1 (*d*)
18	13.6 (*q*)	13.6 (*q*)	12.8 (*q*)	12.8 (*q*)	14.9 (*q*)
19	19.4 (*q*)	19.4 (*q*)	19.4 (*q*)	19.4 (*q*)	19.3 (*q*)
20	71.3 (*d*)	71.4 (*d*)	207.7 (*s*)	207.7 (*s*)	216.1 (*s*)
21	75.0 (*t*)	75.0 (*t*)	77.0 (*t*)	77.2 (*t*)	78.8 (*t*)
21-OMe	59.2 (*q*)	59.2 (*q*)	59.2 (*q*)	59.2 (*q*)	59.2 (*q*)
	Dig	Dig	Dig	Cym	Cym
1′	95.5 (*d*)	95.4 (*d*)	95.6 (*d*)	95.5 (*d*)	95.7 (*d*)
2′	37.0 (*t*)	37.2 (*t*)	37.0 (*t*)	34.0 (*t*)	35.3 (*t*)
3′	66.3 (*d*)	66.3 (*d*)	66.3 (*d*)	77.3 (*d*)	77.0 (*d*)
4′	82.5 (*d*)	82.4 (*d*)	82.4 (*d*)	72.4 (*d*)	82.4 (*d*)
5′	67.9 (*d*)	67.9 (*d*)	68.0 (*d*)	70.7 (*d*)	68.2 (*d*)
6′	18.1 (*q*)	18.1 (*q*)	18.1 (*q*)	18.3 (*q*)	18.2 (*q*)
3′-OMe				57.2 (*q*)	57.8 (*q*)
	Cym	Cym	Cym		Cym
1″	98.0 (*d*)	98.3 (*d*)	98.3 (*d*)		98.6 (*d*)
2″	33.7 (*t*)	35.4 (*t*)	35.4 (*t*)		35.3 (*t*)
3″	77.3 (*d*)	76.7 (*d*)	76.7 (*d*)		77.0 (*d*)
4″	72.2 (*d*)	82.3 (*d*)	82.3 (*d*)		82.5 (*d*)
5″	70.9 (*d*)	68.6 (*d*)	68.6 (*d*)		68.4 (*d*)
6″	18.2 (*q*)	18.1 (*q*)	18.1 (*q*)		18.1 (*q*)
3″-OMe	57.4 (*q*)	58.3 (*q*)	58.3 (*q*)		58.1 (*q*)
		Ole	Ole		Ole
1‴		101.3 (*d*)	101.3 (*d*)		101.3 (*d*)
2‴		35.3 (*t*)	35.3 (*t*)		35.2 (*t*)
3‴		80.5 (*d*)	80.5 (*d*)		80.5 (*d*)
4‴		75.2 (*d*)	75.2 (*d*)		75.2 (*d*)
5‴		71.5 (*d*)	71.5 (*d*)		71.4 (*d*)
6‴		17.9 (*q*)	17.9 (*q*)		17.9 (*q*)
3‴-OMe		56.3 (*q*)	56.3 (*q*)		56.2 (*q*)

H–C(5′) revealed the axial orientation of H–C(4′). The complete assignments of each C-atom signal in this sugar moiety were made by using the HSQC spectrum (*Table 1*). Thus, this sugar unit was identified as digitoxose on the basis of the above data. Similarly, the other sugar unit was characterized as cymarose. The β-linkage of the two sugar units was determined by the large coupling constants ($J = 9.6$ Hz) of each anomeric H-atom signal. The linkage sites of each sugar residue were established on the basis of the HMBC spectrum of **1**, in which ^{1}H,^{13}C long-range correlation signals were observed between H–C(1″) at δ(H) 4.70 (*dd*, $J = 9.6$, 1.6) and C(4′) at δ(C) 82.5, and between H–C(1′) at δ(H) 4.90 (*dd*, $J = 9.6$, 1.6) and C(3) at δ(C) 77.6.

Compound **2** possessed the identical aglycone as **1** according to the ^{13}C-NMR data,

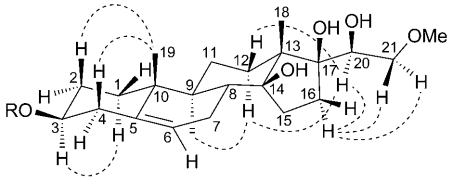


Figure. *Main NOE signals* (dashed lines) *observed in the ROESY spectrum of* **1**

while three sugar units could be observed in its saccharide part. The three sugar residues were identified to be β-digitoxose, β-cymarose, and β-oleandrose by selective 1D-TOCSY and 2D-NMR spectroscopic analyses. The linkage sites of each sugar residue were established on the basis of the HMBC spectrum, in which ^{1}H,^{13}C long-range correlation signals were observed between H–C(1‴) at δ(H) 4.44 (*d*, $J = 9.6$) and C(4″) at δ(C) 82.3; between H–C(1″) at δ(H) 4.75 (*d*, $J = 9.6$) and C(4′) at δ(C) 82.4; and between H–C(1′) at δ(H) 4.86 (*d*, $J = 9.6$) and C(3) at δ(C) 77.5. Further analysis of the ROESY spectrum of **2** confirmed its structure to be the new (3β,14β,17β,20*S*)-3,14,17,20-tetrahydroxy-21-methoxypregn-5-en-3-*O*-β-oleandropyranosyl-(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-digitoxopyranoside, and assigned the trivial name perisepiumoside B.

The ^{13}C-NMR data of **3** were similar to those of **2** except that one oxygenated CH group at δ(C) 71.4 in **2** was replaced by a C=O group at δ(C) 207.7 in **3**. Analyses of the ^{1}H,^{1}H-COSY and HMQC spectra of **3** led to the deduction of the following three fragments in the aglycone part: C(1)–C(2)–C(3)–C(4), C(6)–C(7)–C(8)–C(9)–C(11)–C(12), and C(15)–C(16). Further analyses of the HMBC and ROESY spectra of **3** enabled the identification of the linkage of the fragments described above with the other C-atoms in the aglycone of **3**, and also the relative configuration of its aglycone as (3β,14β,17β)-3,14,17-trihydroxy-21-methyoxypregn-5-en-20-one [9]. Analyses of the ^{1}H-, ^{13}C-NMR, selective 1D-TOCSY, and HMBC spectra of **3** revealed its sugar moiety to be identical to that of **2**. Therefore, the structure of **3** was identified to be (3β,14β,17β)-3,14,17-trihydroxy-21-methoxypregn-5-en-20-one-3-*O*-β-oleandropyranosyl-(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-digitoxopyranoside. It is a new compound and has been given the trivial name perisepiumoside C.

Analysis of the ^{13}C-NMR spectrum of **4** revealed the aglycone to be identical to that of **3**. Only one anomeric H-atom signal could be observed at δ(H) 4.78 (*dd*, $J = 9.4$, 2.0)

Table 2. *^{1}H-NMR Data of* **1**–**5** (400 MHz, in $CDCl_3$)

	1	**2**	**3**	**4**	**5**
H_α–C(1)	1.04–1.09 (*m*)	1.01–1.06 (*m*)	1.01–1.06 (*m*)	1.02–1.07 (*m*)	1.02–1.07 (*m*)
H_β–C(1)	1.83–1.87 (*m*)	1.80–1.84 (*m*)	1.80–1.85 (*m*)	1.80–1.84 (*m*)	1.81–1.86 (*m*)
H_α–C(2)	1.89–1.93 (*m*)	1.84–1.88 (*m*)	1.86–1.90 (*m*)	1.86–1.90 (*m*)	1.89–1.93 (*m*)
H_β–C(2)	1.54–1.57 (*m*)	1.51–1.55 (*m*)	1.52–1.55 (*m*)	1.51–1.54 (*m*)	1.54–1.57 (*m*)
H–C(3)	3.50–3.58 (*m*)	3.47–3.59 (*m*)	3.45–3.52 (*m*)	3.50–3.55 (*m*)	3.49–3.54 (*m*)
H_α–C(4)	2.34–2.38 (*m*)	2.30–2.35 (*m*)	2.30–2.34 (*m*)	2.30–2.35 (*m*)	2.30–2.36 (*m*)
H_β–C(4)	2.17–2.23 (*m*)	2.12–2.16 (*m*)	2.13–2.16 (*m*)	2.13–2.17 (*m*)	2.16–2.19 (*m*)
H–C(6)	5.39 (br. *s*)	5.36 (br. *s*)	5.35 (br. *s*)	5.35 (br. *s*)	5.40 (*d*, *J* = 4.9)
CH_2(7)	2.20–2.24 (*m*)	2.16–2.20 (*m*)	2.16–2.20 (*m*)	2.16–2.20 (*m*)	2.16–2.20 (*m*)
H–C(8)	1.72–1.76 (*m*)	1.72–1.77 (*m*)	1.72–1.76 (*m*)	1.73–1.76 (*m*)	1.72–1.76 (*m*)
H–C(9)	1.05–1.10 (*m*)	1.01–1.05 (*m*)	1.01–1.05 (*m*)	1.01–1.05 (*m*)	1.01–1.05 (*m*)
CH_2(11)	1.48–1.53 (*m*)	1.43–1.48 (*m*)	1.49–1.52 (*m*)	1.48–1.52 (*m*)	1.46–1.50 (*m*)
H_α–C(12)	1.00–1.04 (*m*)	0.96–1.02 (*m*)	1.12–1.16 (*m*)	1.12–1.16 (*m*)	1.48–1.52 (*m*)
H_β–C(12)	1.61–1.64 (*m*)	1.58–1.63 (*m*)	1.26–1.30 (*m*)	1.25–1.30 (*m*)	1.34–1.38 (*m*)
H_α–C(15)	1.96–2.02 (*m*)	1.93–2.01 (*m*)	2.02–2.07 (*m*)	2.01–20.5 (*m*)	2.08–2.13 (*m*)
H_β–C(15)	1.78–1.81 (*m*)	1.74–1.79 (*m*)	1.73–1.78 (*m*)	1.75–1.79 (*m*)	1.73–1.78 (*m*)
H_α–C(16)	1.77–1.82 (*m*)	1.76–1.82 (*m*)	2.64–2.68 (*m*)	2.64–2.69 (*m*)	1.90–1.96 (*m*)
H_β–C(16)	1.78–1.82 (*m*)	1.76–1.80 (*m*)	2.63–2.69 (*m*)	2.63–2.68 (*m*)	1.83–1.87 (*m*)
H–C(17)					2.89 (*dd*, *J* = 3.6, 9.1)
Me(18)	1.19 (*s*)	1.16 (*s*)	1.14 (*s*)	1.14 (*s*)	0.96 (*s*)
Me(19)	0.99 (*s*)	0.95 (*s*)	0.95 (*s*)	0.95 (*s*)	0.95 (*s*)
H–C(20)	3.79–3.83 (*m*)	3.79–3.83 (*m*)			
CH_2(21)	3.54–3.58 (*m*), 3.48–3.53 (*m*)	3.50–3.59 (*m*), 3.45–3.50 (*m*)	4.50 (*d*, *J* = 18.8), 4.30 (*d*, *J* = 18.8)	4.50 (*d*, *J* = 18.8), 4.30 (*d*, *J* = 18.8)	4.12 (*d*, *J* = 19.0), 4.08 (*d*, *J* = 19.0)
MeO–C(21)	3.38 (*s*)	3.34 (*s*)	3.34 (*s*)	3.34 (*s*)	3.34 (*s*)
	Dig	Dig	Dig	Cym	Cym
H–C(1′)	4.90 (*dd*, *J* = 9.6, 1.6)	4.86 (br. *d*, *J* = 9.6)	4.85 (br. *d*, *J* = 9.6)	4.78 (*dd*, *J* = 9.4, 2.0)	4.86 (br. *d*, *J* = 9.6)
CH_2(2′)	2.07–2.13 (*m*), 1.68–1.72 (*m*)	2.00–2.07 (*m*), 1.63–1.68 (*m*)	2.00–2.07 (*m*), 1.63–1.67 (*m*)	2.20–2.26 (*m*), 1.58–1.63 (*m*)	2.07–2.14 (*m*), 1.58–1.62 (*m*)
H–C(3′)	4.22 (br. *s*)	4.18 (br. *s*)	4.18 (br. *s*)	3.62 (br. *s*)	3.80 (br. *s*)
H–C(4′)	3.20 (br. *d*, *J* = 9.4)	3.13 (br. *d*, *J* = 9.5)	3.13 (br. *d*, *J* = 9.5)	3.23 (*dd*, *J* = 9.6, 3.2)	3.20 (br. *d*, *J* = 9.6)
H–C(5′)	3.78 (*dq*, *J* = 9.4, 6.2)	3.70 (*dq*, *J* = 9.5, 6.0)	3.70 (*dq*, *J* = 9.5, 6.0)	3.57 (*dq*, *J* = 9.6, 6.0)	3.85 (*dq*, *J* = 9.6, 6.0)
Me(6′)	1.21 (*d*, *J* = 6.2)	1.18 (*d*, *J* = 6.0)	1.18 (*d*, *J* = 6.0)	1.28 (*d*, *J* = 6.0)	1.19 (*d*, *J* = 6.0)
MeO–C(3′)				3.40 (*s*)	3.41 (*s*)
	Cym	Cym	Cym		Cym
H–C(1″)	4.70 (*dd*, *J* = 9.6, 1.6)	4.75 (br. *d*, *J* = 9.6)	4.75 (br. *d*, *J* = 9.6)		4.75 (br. *d*, *J* = 9.6)
CH_2(2″)	2.18–2.26 (*m*), 1.58–1.62 (*m*)	2.02–2.09 (*m*), 1.53–1.56 (*m*)	2.02–2.08 (*m*), 1.52–1.56 (*m*)		2.02–2.08 (*m*), 1.52–1.56 (*m*)
H–C(3″)	3.62 (br. *s*)	3.75 (br. *s*)	3.75 (br. *s*)		3.80 (br. *s*)
H–C(4″)	3.18 (br. *d*, *J* = 9.4)	3.17 (br. *d*, *J* = 9.5)	3.17 (br. *d*, *J* = 9.5)		3.20 (br. *d*, *J* = 9.5)

Table 2 (cont.)

	1	**2**	**3**	**4**	**5**
H–C(5″)	3.59 (*dq*, *J* = 9.4, 6.4)	3.85 (*dq*, *J* = 9.5, 6.0)	3.85 (*dq*, *J* = 9.5, 6.0)		3.84 (*dq*, *J* = 9.5, 6.0)
Me(6″)	1.24 (*d*, *J* = 6.4)	1.18 (*d*, *J* = 6.0)	1.18 (*d*, *J* = 6.0)		1.18 (*d*, *J* = 6.0)
MeO–C(3″)	3.43 (*s*)	3.40 (*s*)	3.40 (*s*)		3.40 (*s*)
		Ole	Ole		Ole
H–C(1‴)		4.44 (br. *d*, *J* = 9.6)	4.44 (br. *d*, *J* = 9.6)		4.45 (br. *d*, *J* = 9.6)
CH_2(2‴)		2.25–2.31 (*m*), 1.38–1.42 (*m*)	2.24–2.30 (*m*), 1.38–1.42 (*m*)		2.26–2.30 (*m*), 1.40–1.44 (*m*)
H–C(3‴)		3.12 (*dd*, *J* = 9.6, 9.0)	3.12 (*dd*, *J* = 9.6, 9.0)		3.13 (*dd*, *J* = 9.5, 9.0)
H–C(4‴)		3.05 (*dd*, *J* = 9.0, 9.0)	3.06 (*dd*, *J* = 9.0, 9.0)		3.04 (*dd*, *J* = 9.0, 9.0)
H–C(5‴)		3.24 (*dq*, *J* = 9.0, 6.0)	3.25 (*dq*, *J* = 9.0, 6.0)		3.24 (*dq*, *J* = 9.0, 6.0)
Me(6‴)		1.25 (*d*, *J* = 6.0)	1.25 (*d*, *J* = 6.0)		1.25 (*d*, *J* = 6.0)
MeO–C(3‴)		3.34 (*s*)	3.34 (*s*)		3.35 (*s*)

in the ^{1}H-NMR spectrum of **4**, corresponding to the anomeric C-atom signal at δ(C) 95.5 in its ^{13}C-NMR spectrum. The sugar residue was identified to be β-cymarose by further analysis of the 2D-NMR spectra of **4**. The ^{1}H,^{13}C long-range correlation signal between the anomeric H-atom signal and C-atom signal at δ(C) 77.3 ppm in the HMBC spectrum indicated the linkage of the sugar moiety to C(3) of the aglycone. Therefore, the structure of **4** was established to be (3β,14β,17β)-3,14,17-trihydroxy-21-methoxypregn-5-en-20-one-3-*O*-β-cymaropyranoside. It is a new compound and has been named as perisepiumoside D.

Compound **5** was obtained a as white amorphous powder with the molecular formula $C_{43}H_{70}O_{13}$, as deduced from HR-ESI-MS and NMR analyses. The ^{13}C-NMR data of the aglycones of **5** and **3** were similar except that one oxygenated quaternary C-atom signal at δ(C) 93.2 in **3** was replaced by one CH signal at δ(C) 57.1 in **5**. The aglycone of **5** was established to be (3β,14β)-3,14-dihydroxy-21-methoxypregn-5-en-20-one by analyses of the ^{1}H,^{1}H-COSY, HMQC, HMBC, and ROESY spectra [16]. The ^{1}H-NMR and ^{13}C-NMR of **5** revealed the existence of three sugar residues in its structure, which were identified as one β-digitoxose and two β-cymarose units by 1D-TOCSY and 2D-NMR analyses using the method mentioned above. In the HMBC spectrum of **5**, ^{1}H,^{13}C long-range correlation signals were found between H–C(1‴) at δ(H) 4.45 (*d*, *J* = 9.6) and C(4″) at δ(C) 82.5; between H–C(1″) at δ(H) 4.75 (*d*, *J* = 9.6) and C(4′) at δ(C) 82.4; and between H–C(1′) at δ(H) 4.86 (*d*, *J* = 9.6) and C(3) at δ(C) 77.5. Therefore, the structure of **5** was determined to be (3β,14β)-3,14-dihydroxy-21-methoxypregn-5-en-20-one-3-*O*-β-oleandropyranosyl-(1 → 4)-*O*-β-cymaropyranosyl-(1 → 4)-*O*-β-cymaropyranoside. It is a new compound and has been named as perisepiumoside E.

In addition, seven known pregnane glycosides were isolated and characterized as periplocoside N (**6**) [7], periplocoside M (**7**) [7], periplocogenin (**8**) [5], (3β,14β,17β)-

3,14,17-trihydroxy-21-methoxypregn-5-en-20-one (**9**) [9], (3*β*,14*β*,17*β*)-3,14,17,20-tetrahydroxy-21-methoxypregn-5-ene (**10**) [9], glycoside K (**11**) [10], and periplocoside E (**12**) [7]. All the known compounds have already been isolated from this plant.

Experimental Part

General. Column chromatography (CC): silica gel *H60* (*Qingdao Haiyang Chemical Group Corporation*, Qingdao, P. R. China). Prep. HPLC: *Varian SD-1* instrument equipped with a *RP-C_{18}* column (12 μm, *Merck NW25*, 20 mm × 250 mm, 10 ml/min) and *Unimicro Technologies ELSD-UM3000* Detector (40°, 2.0 bar). A split valve was used to connect the outlet of the *RP-C_{18}* column with the inlet of the ELSD system and the pipeline of the fraction collector. Ten percent of the effluent from the outlet of the column were detected by ELSD, and the rest was collected simultaneously in a fraction collector. TLC: *HSG_{254}* silica gel plates (*Yantai Chemical Industrial Institute*, Yantai, P. R. China). Optical rotation: *Perkin-Elmer 241MC* polarimeter. IR Spectra: *Perkin-Elmer 577* spectrometer; in cm^{-1}. NMR Spectra: *Bruker AM-400* spectrometer; δ in ppm rel to Me_4Si as internal standard, J in Hz. HR-ESI-MS: *Micromass LCT* spectrometer; in *m/z*.

Plant Material. The root barks of *Periploca sepium* were purchased from the Shanghai Hua-Yu Herb Medicine Cooperation in 2001, and identified by Prof. *Jingui Shen* of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences. A voucher specimen was deposited with the Herbarium of the Shanghai Institute of Materia Medica (No. 20010605).

Extraction and Isolation. The dried root barks of *Periploca sepium* (15.0 kg) were extracted with 95% EtOH (10.0 l × 3) at reflux for 2 h each time. After concentration, the residue was suspended in H_2O (2.0 l), and then extracted with $CDCl_3$ (2.0 l × 3) and BuOH (2.0 l × 3) successively, yielding a $CDCl_3$ extract (762.5 g) and BuOH extract (75.2 g), resp.

The $CDCl_3$ extract (762.5 g) was subjected to CC (SiO_2; petroleum ether (PE)/acetone 5 : 1 → 1 : 2) to give fractions 1 – 4. *Fr. 2* (15.8 g) was separated using prep. HPLC (*RP-18*; MeOH/H_2O 5 : 5 → 9 : 1) to give **1** (180.4 mg), **2** (71.8 mg), **3** (29.7 mg), **4** (10.1 mg), **5** (30.7 mg), periplocoside N (1.3 g) (**6**), periplocoside M (792.3 mg) (**7**), periplocoside E (1.2 g) (**12**), periplocogenin (6.2 mg) (**8**), (3*β*, 14*β*,17*β*)-3,14,17-trihydroxy-21-methoxypregn-5-en-20-one (20.1 mg) (**9**), and (3*β*,14*β*,17*β*)-3,14,17,20-tetrahydroxy-21-methoxypregn-5-ene (11.0 mg) (**10**). The BuOH extract (2.0 g) was subjected to prep. HPLC (*RP-18*; MeOH/H_2O 1 : 9 → 10 : 0) to afford glycoside K (42.8 mg) (**11**).

Perisepiumoside A (= *(3β,14β,17β,20*S*)-3,14,17,20-Tetrahydroxy-21-methoxypregn-5-ene-3*-O-*β*-*cymaropyranosyl-(1 → 4)*-O-*β*-*digitoxopyranoside*, **1**). White amorphous powder. $[\alpha]_D^{24} = -5.0$ ($c = 0.11$, $CHCl_3$). IR (KBr): 3448, 2933, 1637, 1452, 1383, 1164, 1078, 1089, 1070, 1003, 729. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 677.3891 ($[M + Na]^+$, $C_{35}H_{58}NaO_{11}^+$; calc. 677.3877).

Perisepiumoside B (= *(3β,14β,17β,20*S*)-3,14,17,20-Tetrahydroxy-21-methoxypregn-5-en-3*-O-*β*-*oleandropyranosyl-(1 → 4)*-O-*β*-*cymaropyranosyl-(1 → 4)*-O-*β*-*digitoxopyranoside*, **2**). White amorphous powder. $[\alpha]_D^{24} = -31.0$ ($c = 0.12$, $CHCl_3$). IR (KBr): 3450, 2935, 1631, 1452, 1369, 1164, 1070, 1089, 1070, 1001, 908, 731. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 821.4663 ($[M + Na]^+$, $C_{42}H_{70}NaO_{14}^+$; calc. 821.4663).

Perisepiumoside C (= *(3β,14β,17β)-3,14,17-Trihydroxy-21-methoxypregn-5-en-20-one-3*-O-*β*-*oleandropyranosyl-(1 → 4)*-O-*β*-*cymaropyranosyl-(1 → 4)*-O-*β*-*digitoxopyranoside*, **3**). White amorphous powder. $[\alpha]_D^{24} = -46.0$ ($c = 0.11$, $CHCl_3$). IR (KBr): 3446, 2935, 1724, 1637, 1452, 1369, 1164, 1060, 1070, 1001, 731. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 819.4057 ($[M + Na]^+$, $C_{42}H_{68}NaO_{14}^+$; calc. 819.4057).

Perisepiumoside D (= *(3β,14β,17β)-3,14,17-Trihydroxy-21-methoxypregn-5-en-20-one-3*-O-*β*-*cymaropyranoside*, **4**). White amorphous powder. $[\alpha]_D^{24} = -34.0$ ($c = 0.05$, $CHCl_3$). IR (KBr): 3504, 2973, 2935, 1735, 1637, 1371, 1243, 1164, 1095, 1056, 1004, 729. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 545.3090 ($[M + Na]^+$, $C_{29}H_{46}NaO_8^+$; calc. 545.3090).

Perisepiumoside E (= *(3β,14β)-3,14-Dihydroxy-21-methoxypregn-5-en-20-one-3*-O-*β*-*oleandropyranosyl-(1 → 4)*-O-*β*-*cymaropyranosyl-(1 → 4)*-O-*β*-*cymaropyranoside*, **5**). White amorphous powder.

$[\alpha]_D^{24} = -1.0$ ($c = 0.11$, $CHCl_3$). IR (KBr): 3448, 2933, 1710, 1637, 1452, 1367, 1164, 1103, 1058, 1003, 729. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS : 817.4717 ($[M+Na]^+$, $C_{43}H_{70}NaO_{13}^+$; calc. 817.4714).

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