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Studies on Chemical Constituents of Antitumor Fraction from *Periploca sepium*. IV. Structures of New Pregnane Glycosides, Periplocosides D, E, L, and M

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Four new pregnane glycosides and a known pregnane glycoside (named periplocoside D, E, L, M, and N, respectively), as well as a steroidal compound S-20, have been isolated from the antitumor fraction of *Periploca sepium* (Asclepiadaceae). Their structures were established by chemical and spectroscopic methods. S-20 was obtained from this plant for the first time.

Keywords——*Periploca sepium*; Asclepiadaceae; pregnane glycoside; periplocoside; ^{13}C-NMR

In our previous papers,[1-3] it has been reported that three new pregnane glycosides, A, B and C, and other related compounds were isolated from the antitumor fraction of *Periploca sepium* (Asclepiadaceae), and their structures were clarified with the aid of two-dimensional nuclear magnetic resonance (2D-NMR) studies. The present paper describes the isolation and structural elucidation of six hydrolysis products of periplocoside A, four new pregnane glycosides S-12, S-13, S-14 and S-15, and a known pregnane glycoside S-11 (named periplocoside M, D, E, L, and N, respectively), and a steroidal compound S-20 which was obtained for the first time from this plant.

On partial hydrolysis with 0.001 N H_2SO_4 in MeOH at room temperature, periplocoside A (**3**)[2] gave six products, namely S-2A (**8**) (3β-*O*-(4′,6′-dideoxy-3′-*O*-methyl-$\Delta^{3'}$-D-2′-hexosulosyl)-Δ^5-pregnene-3β,17α,20(*S*)-triol),[1] S-12 (**5**), and compounds **11**, **12**, **13** and **14**. In the proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra of **11** and **12**, both showed signals of a dioxymethylene group (δ 4.72 and 96.98) linked to the C-3 of canarose. It was presumed that **11** contained 3,4-methylenedioxycanarose and **12** contained 3-*O*-methoxymethylcanarose from the chemical shifts of the C-3 and C-4 carbon signals due to the canarose. Consequently, **11** and **12** were deduced to be Δ^5-pregnene 3β,17α,20(*S*)-triol 3-*O*-(4′,6′-dideoxy-3′-*O*-methyl-$\Delta^{3'}$-D-2′-hexosuloside) 20-*O*-(3′′,4′′-methylenedioxy-β-D-canaropyranoside) and Δ^5-pregnene-3β,17α,20(*S*)-triol 3-*O*-(4′,6′-dideoxy-3′-*O*-methyl-$\Delta^{3'}$-D-2′-hexosuloside) 20-*O*-(3′′-*O*-methoxymethyl-β-D-canaropyranoside) respectively. The ^{1}H-, ^{13}C-NMR and infrared (IR) spectra of **13** suggested the presence of five methyl groups (δ 1.16, 1.19, 1.21, 1.36 and 1.44), five methoxyl groups (δ 3.37, 3.41, 3.43, 3.44 and 3.46), one acetyl group (δ 20.96 and 169.35), one lactone (δ 168.07 and ν 1750 cm^{-1}) and four sugar anomeric proton and carbon signals (δ 99.63, 99.79 ($\times$2), 102.55). By comparison of its spectral data with those of **3** and D-oleandronic-δ-lactone,[4] **13** was established to be 2-*O*-acetyl-β-D-digitalopyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-oleandronic-δ-lactone. Also, the structure of **14** was deduced to be methyl 2-*O*-acetyl-β-D-digitalopyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranoside. These products were presumably produced from **3** by the partial hydrolytic mechanism shown in Fig. 1. Consequently, the structure of **3** was also further supported by the above results.

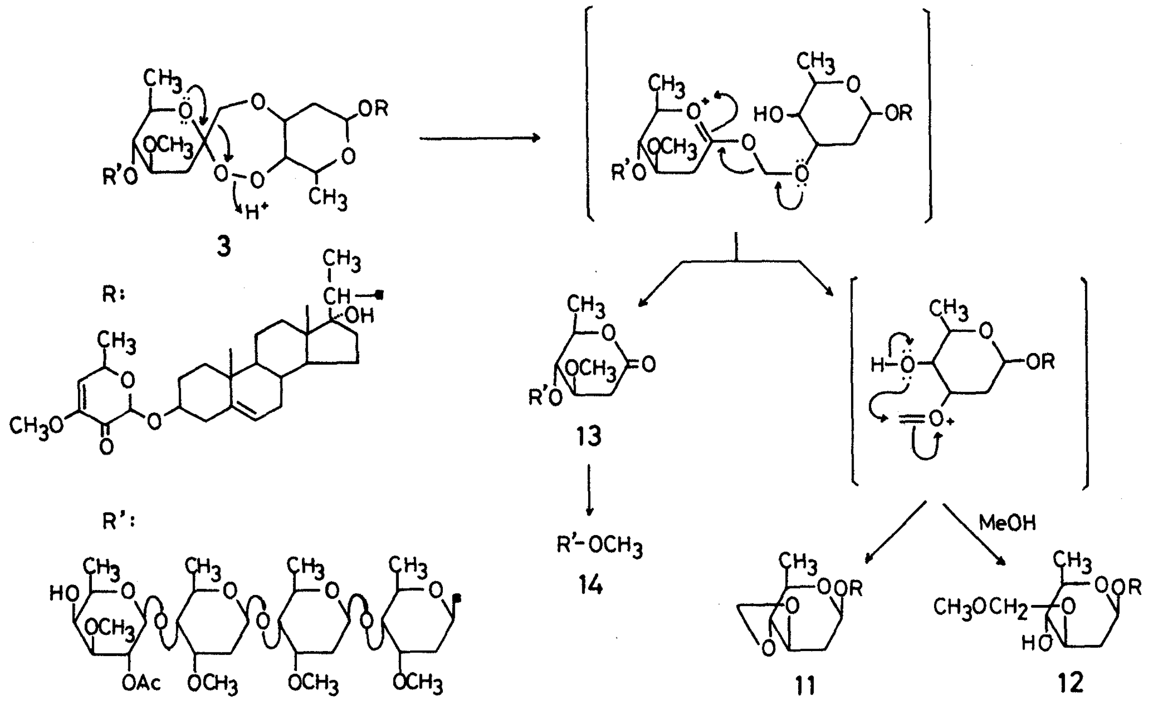


Fig. 1. Partial Hydrolysis of Periplocoside A (3)

Periplocoside D (1), colorless powder, mp 191—193 °C and $[\alpha]_D$ $-3.08°$ (c=0.26, $CHCl_3$), was hydrolyzed with 0.05 N H_2SO_4 in 50% aqueous MeOH to give **8**, cymarose, canarose and digitalose, which were identified by direct comparison with authentic samples on thin layer chromatography (TLC). The ^{13}C-NMR spectrum of **1** showed five doublet signals due to anomeric carbons of three cymarose (δ 98.49, 99.70 ($\times$2)), one canarose (δ 100.81) and one digitalose (δ 104.61) moiety, and two characteristic signals due to 3,7-dideoxy-4-O-methyl-α-D-gluco-2-heptulopyranose at δ 113.72 (s) and δ 86.37 (t) other than signals due to **8**, but did not show the signals due to the acetyl group of 2-O-acetyl-D-digitalose in the terminal sugar of the sugar chain (Table I). Also, a positive color reaction for peroxide[5] was observed. Compound **1** was acetylated with Ac_2O/pyridine at room temperature to afford its diacetate **4**, whose 2 and 4-proton signals due to the digitalose moiety were shifted downfield at δ 5.11 (dd, J=10.1; 8.0 Hz) and 5.31 (dd, J=3.2, 1.2 Hz), respectively, in comparison with those of **3**. Further, the physical and spectral data of **4** were identical to those of periplocoside A′ (**4**) which was prepared by acetylating **3** with Ac_2O/pyridine.[2] From the above results, **1** was confirmed to be Δ^5-pregnene-3β,17α,20(S)-triol 3-O-(4′,6′-dideoxy-3′-O-methyl-$\Delta^{3'}$-D-2′-hexosuloside) 20-O-(β-D-digitalopyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranosyl(1→5)-3,7-dideoxy-4-O-methyl-α-D-gluco-2-heptulopyranosyl(2-4)-dioxy-(1→3)-β-D-canaropyranoside).

Periplocoside E (**2**), colorless powder, mp 183—185 °C and $[\alpha]_D$ $-7.5°$ (c=0.08, MeOH), was hydrolyzed in the same way as described for **1**. The hydrolyzate consisted of Δ^5-pregnene-3β,17α,20(S)-triol (**9**),[6] cymarose, canarose and digitalose by direct TLC comparison with authentic samples. The ^1H- and ^{13}C-NMR signals of **2** were similar to those of **3** except for the signals due to 4′,6′-dideoxy-3′-O-methyl-$\Delta^{3'}$-D-2′-hexosulose linked to the C-3 hydroxyl group of the genin. Also, a positive color reaction for peroxide was detected, and glycosylation shifts in the ^{13}C-NMR spectrum of **2** were observed at the C-3 (+6.88 ppm), C-2 (−2.50 ppm) and C-4 (−3.69 ppm) carbon signals in comparison with that of **3**. Consequently, **2** was deduced to be Δ^5-pregnene-3β,17α,20(S)-triol 20-O-(2-O-acetyl-β-D-digitalopyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaropyranosyl(1→4)-β-D-cymaro-

TABLE I. ^{13}C-NMR Chemical Shifts of Periplocosides D (**1**), E (**2**), A′ (**4**), M (**5**), N (**6**), **9** and S-20 (**10**)

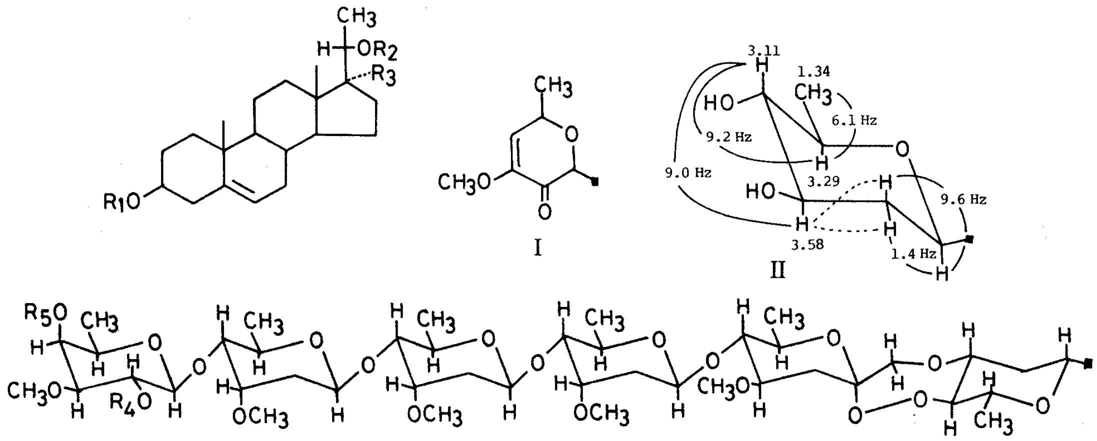
		D	E	A′	N	M′	S-20	9	L
1	t	37.37	37.23	37.35	37.24	37.37	38.17	37.29	37.35
2	t	29.41	31.89	29.39	31.93	29.41	27.80	31.67	29.64
3	d	78.61	71.73	78.58	71.74	78.61	74.00	71.75	78.64
4	t	38.55	42.24	38.55	42.24	38.55	37.05	42.31	38.91
5	s	140.34	140.71	140.35	140.72	140.34	139.81	140.73	140.80
6	d	122.00	121.63	121.98	121.63	122.00	122.48	121.60	121.84
7	t	31.91	31.89	31.90	31.86	31.93	31.96	31.91	31.96
8	d	31.91	31.63	31.90	31.61	31.93	31.77	31.91	31.89
9	d	49.72	49.66	49.69	49.63	49.72	50.10	49.71	49.77
10	s	36.74	36.78	36.90	36.48	36.74	36.77	36.52	36.78
11	t	20.58	20.59	20.56	20.59	20.58	21.42	20.57	20.55
12	t	36.92	36.94	36.90	39.35	39.41	39.89	37.72	31.11
13	s	45.36	45.35	45.34	45.35	45.37	42.32	45.70	45.71
14	d	51.11	51.09	51.09	51.10	51.13	58.54	51.44	51.46
15	t	23.47	23.48	23.45	23.48	23.49	25.68	23.57	23.57
16	t	30.99	30.99	30.97	30.97	31.01	24.58	31.11	37.72
17	s	85.46	85.47	85.45	85.48	85.48	56.20 (d)	85.77	85.77
18	q	14.14	14.14	14.12	14.14	14.14	12.39	14.04	14.04
19	q	19.35	19.35	19.33	19.39	19.36	19.34	19.40	19.39
20	d	83.06	82.91	83.05	82.90	82.97	70.61	72.37	72.37
21	q	17.99	18.00	17.98	17.79	17.76	23.69	18.62	18.62
1′	d	97.29		97.28		97.29	170.53	Digitalose	
2′	s	185.88		185.85		185.91	(s, OAc)	1	101.20 (d)
3′	s	147.84		147.85		147.84	20.94	2	70.63 (d)
4′	d	118.49		118.47		118.50	(q, OAc)	3	82.96 (d)
5′	d	68.87		68.85		68.89		4	68.08 (d)
6′	q	23.01		22.99		23.01		5	70.43 (d)
OMe	q	54.97		54.95		54.98		6	16.50 (q)
Canarose								OMe	57.44 (q)
1	d	100.81	100.82	100.80	100.85	100.80			
2	t	38.40	38.40	38.40	38.36	38.42			
3	d	78.30	78.31	78.28	71.65	71.62			
4	d	79.20	79.21	79.19	77.45	77.58			
5	d	69.98	70.00	69.96	71.74	71.82			
6	q	17.05	17.07	17.04	17.00	17.02			
Heptulose									
1	t	86.37	86.39	86.37					
2	s	113.72	113.70	113.70					
3	t	36.74	36.72	36.72					
4	d	77.04	77.03	76.97					
5	d	82.63	82.64	82.55					
6	d	69.81	69.86	69.79					
7	q	17.99	18.00	17.98					
OMe	q	57.67	57.70	57.76					

		D	E	A′
Cymarose (1)				
1	d	98.49	98.50	98.48
2	t	36.73	36.70	36.72
3	d	77.67	77.60	77.65
4	d	82.49	82.51	82.55
5	d	68.87	68.86	68.85
6	q	18.23	18.25	18.22
OMe	q	58.04	57.97	57.92
Cymarose (2)				
1	d	99.70	99.75	99.73
2	t	35.55	35.56	35.55
3	d	77.60	77.58	77.65
4	d	82.49	82.51	82.49
5	d	68.51	68.45	68.49
6	q	18.23	18.25	18.22
OMe	q	58.04	58.04	58.03
Cymarose (3)				
1	d	99.70	99.75	99.73
2	t	35.45	35.47	35.25
3	d	77.36	77.34	77.64
4	d	82.82	82.90	83.91
5	d	68.45	68.20	68.05
6	q	18.23	18.25	18.22
OMe	q	58.18	58.66	58.18
Digitalose				
1	d	104.61	102.55	102.54
2	t	70.36	70.93	70.89
3	d	83.06	81.60	80.05
4	d	68.08	68.13	68.40
5	d	70.76	70.39	69.27
6	q	16.49	16.51	16.53
OMe	q	57.67	57.42	57.66
OAc	s		169.47	169.34
	q		20.99	20.96
OAc	s			170.85
	q			20.79

The measurements were made on a Bruker AM400 instrument in $CDCl_3$ with TMS as an internal reference and are expressed in terms of ppm. Assignments of methoxyl groups due to canarose, heptulose, cymarose and digitalose may be interchanged.

pyranosyl(1→5)-3,7-dideoxy-4-*O*-methyl-α-D-gluco-2-heptulopyranosyl(2-4)-dioxy-(1→3)-β-D-canaropyranoside).

Periplocoside M (**5**), colorless needles, mp 195—197 °C and $[\alpha]_D$ −89.91° (c=0.23, MeOH), was hydrolyzed in the same way as described for **1** to give **8**. The ^{1}H- and ^{13}C-NMR spectra of **5** showed only one anomeric signal due to 2,6-dideoxy sugar at δ 4.61 (dd, J=9.6, 1.4 Hz, β-linkage) and 100.81 other than the signals due to **8**. Its sugar was deduced to be β-D-



III

periplocoside D (**1**): $R_1=I$, $R_2=III$, $R_3=OH$, $R_4=H$, $R_5=H$
periplocoside E (**2**): $R_1=H$, $R_2=III$, $R_3=OH$, $R_4=Ac$, $R_5=H$
periplocoside A (**3**): $R_1=I$, $R_2=III$, $R_3=OH$, $R_4=Ac$, $R_5=H$
periplocoside A′ (**4**): $R_1=I$, $R_2=III$, $R_3=OH$, $R_4=Ac$, $R_5=Ac$
periplocoside M (**5**): $R_1=I$, $R_2=II$, $R_3=OH$
periplocoside N (**6**): $R_1=H$, $R_2=II$, $R_3=OH$
periplocoside L (**7**): $R_1=$digitalose, $R_2=H$, $R_3=OH$
S-2A (**8**): $R_1=I$, $R_2=H$, $R_3=OH$
(**9**): $R_1=H$, $R_2=H$, $R_3=OH$
S-20 (**10**): $R_1=Ac$, $R_2=H$, $R_3=H$

Chart 1

canarose from the correlated spin–spin couplings as shown in (II) of Chart 1. The glycosylation shift was observed at the C-20 (+10.62 ppm) carbon signal. Based on these results, **5** was concluded to be Δ^5-pregnene-3β,17α,20(*S*)-triol 3-*O*-(4′,6′-dideoxy-3′-*O*-methyl-$\Delta^{3'}$-D-2′-hexosuloside) 20-*O*-β-D-canaropyranoside.

Periplocoside N (**6**), colorless needles, mp 236—238 °C and $[\alpha]_D$ −70.5° (c=0.18, MeOH), was hydrolyzed to yield **9** and canarose. The ^{1}H- and ^{13}C-NMR spectra of **6** did not exhibit signals due to the hexosulose at the C-3 hydroxyl group of its aglycone. Treatment of **5** with 0.4 N NaOH under a nitrogen atmosphere for 30 min afforded **6** which gave the expected ^{1}H- and ^{13}C-NMR spectral data. Therefore, **6** was concluded to be Δ^5-pregnene-3β,17α,20(*S*)-triol 20-*O*-β-D-canaropyranoside, which is a known compound.[7]

Periplocoside L (**7**), colorless needles, mp 238—240 °C and $[\alpha]_D$ −53.3° (c=0.06, MeOH), was hydrolyzed to furnish **9** and digitalose. A comparison of the ^{13}C-NMR signals of **7** with those of **9** revealed glycosylation shifts at C-3 (+6.89 ppm), C-2 (−2.04 ppm) and C-4 (−3.40 ppm) in **7**. Therefore, **7** was determined to be Δ^5-pregnene-3β,17α,20(*S*)-triol 3-*O*-β-D-digitalopyranoside. This compound is of interest in connection with the biosynthetic conversion to S-2A, because digitaloside was converted into 4,6-dideoxy-3-*O*-methyl-Δ^3-2-hexosuloside according to Cruz *et al.*[8] and Abe *et al.*[9]

S-20 (**10**), colorless needles, mp 165—167 °C and $[\alpha]_D$ −65.2° (c=1.2, $CHCl_3$), electron impact-mass spectra (EI-MS) *m/z*: 300 [$M^+(C_{23}H_{36}O_3)$−AcOH], has one double bond (δ 5.36) at C-5 and one acetyl group linked to the C-3 hydroxyl group of the pregnene skeleton, as judged from its ^{1}H- and ^{13}C-NMR spectra, and was established to be Δ^5-pregnene-3β,20(*R*)-diol 3-*O*-monoacetate.[10] This is the first report of its isolation from this plant.

Experimental

All melting points were recorded on a Yanagimoto micro melting point apparatus and are uncorrected. The

NMR spectra were taken on a Bruker AM 400 instrument at 400 MHz for ^1H and 100.6 MHz for ^{13}C, and chemical shifts are given as δ (ppm) with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet). MS were measured on a Hitachi M-80 spectrometer.

The following solvent systems were used with 0.25 mm Kieselgel F_{254} (Merck) TLC plates: solvent 1, $CHCl_3$–MeOH (96:4); solvent 2, $CHCl_3$–MeOH–H_2O (7:3:1) lower phase; solvent 3, $CHCl_3$–MeOH (9:1) and solvent 4, $CHCl_3$–Me_2CO (2:1). Each spot on the TLC plate was detected by spraying 10% H_2SO_4 and heating the plate.

Partial Hydrolysis of Periplocoside A (3)——A solution of **3** in 0.001 N H_2SO_4–MeOH (3 ml) was stirred for 30 min at room temperature. The reaction mixture was diluted with H_2O (10 ml) and extracted with EtOAc. The extract was purified by means of silica gel column chromatography using EtOAc as the eluent to give S-2A (**8**),[1] S-12 (**5**), **11**, **12**, **13** and **14**.

Compound **11**: ^1H-NMR ($CDCl_3$) δ: 0.73 (3H, s, C-18), 1.00 (3H, s, C-19), 1.30 (3H, d, J=6.5 Hz, C-21), 1.51 (6H, d, J=6.0 Hz, C-6′ and can-6), 3.63 (3H, s, C-3′OMe), 3.74 (1H, q, J=6.5 Hz, C-20), 4.58 (1H, dd, J=10, 1.5 Hz, can-1), 4.72 (2H, s, $-OCH_2O-$), 5.05 (1H, s, C-1′), 5.78 (1H, d, J=3.0 Hz, C-4′). ^{13}C-NMR ($CDCl_3$) δ: 14.15 (q, C-18), 17.01 (q, can-6), 18.07 (q, C-21), 19.36 (q, C-19), 20.59 (t, C-11), 23.03 (q, C-6′), 23.49 (t, C-15), 29.41 (t, C-2), 31.02 (t, C-16), 31.49 (t, C-7 and d, C-8), 36.74 (s, C-10), 37.38 (t, C-1), 37.76 (t, can-2), 38.44 (t, C-12), 38.52 (t, C-4), 45.38 (s, C-13), 49.74 (d, C-9), 51.14 (d, C-14), 54.94 (q, OMe), 68.89 (d, C-5′), 71.79 (d, can-5), 78.32 (d, can-3), 78.64 (d, C-3), 79.32 (d, can-4), 82.86 (d, C-20), 85.48 (s, C-17), 96.98 (t, $-OCH_2O-$), 97.32 (d, C-1′), 100.77 (d, can-1), 118.49 (d, C-4′), 121.92 (d, C-6), 140.36 (s, C-5), 147.84 (s, C-3′), 185.91 (s, C-2′).

Compound **12**: ^1H-NMR ($CDCl_3$) δ: 0.73 (3H, s, C-18), 1.01 (3H, s, C-19), 1.30 (3H, d, J=6.5 Hz, C-21), 1.51 (6H, d, J=6.0 Hz, C-6′ and can-6), 3.49 (3H, s, OMe), 3.63 (3H, s, C-3′OMe), 3.74 (1H, q, J=6.5 Hz, C-20), 4.58 (1H, dd, J=10, 1.5 Hz, can-1), 4.72 (2H, s, $-OCH_2O-$), 5.05 (1H, s, C-1′), 5.78 (1H, d, J=3.0 Hz, C-4′). ^{13}C-NMR ($CDCl_3$) δ: 14.15 (q, C-18), 17.01 (q, can-6), 18.07 (q, C-21), 19.36 (q, C-19), 20.59 (t, C-11), 23.03 (q, C-6′), 23.49 (t, C-15), 29.41 (t, C-2), 31.02 (t, C-16), 31.94 (t, C-7 and d, C-8), 36.74 (s, C-10), 37.38 (t, C-1), 37.76 (t, can-2), 38.44 (t, C-12), 38.52 (t, C-4), 45.38 (s, C-13), 49.74 (d, C-9), 51.14 (d, C-14), 54.97 (q, C-3′OMe), 55.74 (q, OMe), 68.89 (d, C-5′), 71.79 (d, can-5), 75.35 (d, can-4), 78.64 (d, C-3), 81.45 (d, can-3), 82.86 (d, C-20), 85.48 (s, C-17), 96.98 (t, $-OCH_2O-$), 97.32 (d, C-1′), 100.80 (d, can-1), 118.49 (d, C-4′), 121.96 (d, C-6), 140.37 (s, C-5), 147.84 (s, C-3′), 185.91 (s, C-2′).

Compound **13**: White powder, mp 148—150 °C, $[\alpha]_D$ +38.2° (c=0.21, $CHCl_3$). ^1H-NMR ($CDCl_3$) δ: 1.16, 1.19, 1.21 (3H, J=6.1 Hz, cym-6, respectively), 1.36 (3H, J=6.5 Hz, dig-6), 1.44 (3H, d, J=6.4 Hz, lactone-6), 2.07 (3H, s, OAc), 2.71 (2H, d, J=3.4 Hz, lactone-2), 3.37, 3.41, 3.43, 3.44, 3.46 (3H, s, OMe, respectively), 3.97 (1H, m, lactone-3), 4.38 (1H, d, J=8.0 Hz, dig-1), 4.40 (1H, dq, J=9.5, 6.4 Hz, lactone-5), 4.74, 4.76, 4.88 (1H, dd, J=10, 1.5 Hz, cym-1, respectively), 5.07 (1H, dd, J=10, 8.0 Hz, dig-2). ^{13}C-NMR ($CDCl_3$) δ: 16.49 (q, dig-6), 18.00, 18.06, 18.24 (q, cym-6, respectively), 19.02 (q, lactone-6), 20.96 (q, OAc), 32.90 (t, lactone-2), 35.40, 35.67, 36.03 (t, cym-2, respectively), 56.81 (s, lactone-3OMe), 57.40 (s, dig-3OMe), 58.05, 58.34, 58.61 (s, cym-3OMe, respectively), 68.05 (1H, d, dig-4), 68.14, 68.50, 68.86 (d, cym-5, respectively), 70.41 (×2) (d, dig-5 and lactone-5), 70.96 (d, dig-2), 76.14, 76.70, 77.34 (d, cym-3, respectively), 78.15 (d, lactone-3), 80.60 (d, lactone-4), 81.59 (d, dig-3), 82.30, 82.50, 83.61 (d, cym-4, respectively), 99.63, 99.78 (×2) (d, cym-1, respectively), 102.55 (d, dig-1), 168.02 (s, lactone-1), 169.35 (s, OAc).

Compound **14**: ^1H-NMR ($CDCl_3$) δ: 1.17, 1.22, 1.23 (3H, d, J=6.2 Hz, cym-6, respectively), 1.37 (3H, d, J=6.5 Hz, dig-6), 2.07 (3H, s, OAc), 3.41×2, 3.43, 3.45, 3.46 (3H, s, OMe, respectively), 4.38 (1H, d, J=8.0 Hz, dig-1), 4.62, 4.73, 4.77 (1H, dd, J=10, 1.5 Hz, cym-1, respectively), 5.08 (1H, dd, J=10, 8.0 Hz, dig-2).

Isolation of S-11, 12, 13, 14, 15 and 20——As we reported in the previous paper,[1] the CM-1 fraction (20 g) was subjected to chromatography on silica gel and eluted with $CHCl_3$–MeOH (9:1) and (5:1). The obtained $CHCl_3$–MeOH (9:1) fraction was separated by silica gel column chromatography using EtOAc as the eluent to afford S-20 (7 mg). The obtained $CHCl_3$–MeOH (5:1) fraction was submitted to HPLC on an RP-18 column and eluted with MeOH–H_2O (8:2) and (7:3) to furnish S-11 (18 mg), S-12 (30 mg), S-13 (45 mg), S-14 (18 mg) and S-15 (2 mg), named periplocosides N, M, D, E and L, respectively.

Periplocoside D (**1**): Colorless powder, mp 191—193 °C, $[\alpha]_D$ −3.08° (c=0.26, $CHCl_3$). ^1H-NMR ($CDCl_3$) δ: 0.72 (3H, s, C-18), 0.99 (3H, s, C-19), 1.19, 1.21, 1.23 (3H, d, J=6.5 Hz, cym-6, respectively), 1.29 (3H×2, d, J=6.5 Hz, C-21 and hep-7), 1.32 (3H, d, J=6.0 Hz, can-6), 1.36 (3H, d, J=6.5 Hz, dig-6), 1.50 (3H, d, J=6.5 Hz, C-6′), 3.43 (×2), 3.44 (×2), 3.51 (3H, s, OMe, respectively), 3.63 (3H, s, C-3′OMe), 4.27 (1H, d, J=8.0 Hz, dig-1), 4.57 (1H, dd, J=9.5, 1.5 Hz, can-1), 4.70 (1H, dq, J=6.8, 3.0 Hz, C-5′), 4.74 (1H, d, J=7.6 Hz, hep-1a), 4.76 (×2) (1H, dd, J=10, 1.5 Hz, cym-1, respectively), 4.92 (1H, dd, J=10, 1.5 Hz), 5.04 (1H, s, C-1′), 5.13 (1H, d, J=7.5 Hz, hep-1b), 5.36 (1H, br s, C-6), 5.77 (1H, d, J=3.0 Hz, C-4′). The acetylation of **1** was carried out in the usual way to afford **4** which was identical with periplocoside A′.[2]

Periplocoside E (**2**): Colorless powder, mp 183—189 °C, $[\alpha]_D$ −7.5° (c=0.08, $CHCl_3$). ^1H-NMR ($CDCl_3$) δ: 0.73 (3H, s, C-18), 1.02 (3H, s, C-19), 1.17, 1.19, 1.21 (3H, d, J=6.5 Hz, cym-6, respectively), 1.29 (×2) (3H, d, J=6.5 Hz, C-21 and hep-7), 1.31 (3H, d, J=6.0 Hz, can -6), 1.37 (3H, d, J=6.5 Hz, dig-6), 2.07 (3H, s, OAc), 3.41, 3.42, 3.43, 3.44 (×2) (3H, s, OMe, respectively), 4.38 (1H, d, J=6.5 Hz, dig-1), 4.57 (1H, dd, J=9.5, 1.5 Hz, can-1), 4.74 (1H, d, J=7.5 Hz, hep-1a), 4.76 (×2) (1H, dd, J=10, 1.5 Hz, cym-1, respectively), 4.92 (1H, dd, J=10, 1.5 Hz, cym-1), 5.08

(1H, dd, J=8.0, 9.75 Hz, dig-2), 5.13 (1H, d, J=7.5 Hz,hep-1b), 5.40 (1H, br s, C-6).

Periplocoside M (**5**): Colorless needles, mp 195—197 °C, $[\alpha]_D$ −89.91 ° (c=0.23, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H, s, C-18), 1.00 (3H, s, C-19), 1.30 (3H, d, J=6.3 Hz, C-21), 1.34 (3H, d, J=6.1 Hz, can-6), 1.51 (3H, d, J=6.8 Hz, C-6′), 3.63 (3H, s, C-3′OMe), 3.66 (1H, m, C-3), 3.74 (1H, q, J=6.3 Hz, C-20), 4.61 (1H, dd, J=9.6, 1.4 Hz, can-1), 4.71 (1H, dq, J=6.8, 3.0 Hz, C-5′), 5.05 (1H, s, C-1′), 5.36 (1H, br s, C-6), 5.78 (1H, d, J=3.0 Hz, C-4′). A solution of **5** (10 mg) in 0.4 N NaOH–MeOH (1.5 ml) was stirred at room temperature for 30 min under an N_2 gas flow. The reaction mixture was neutralized with Amberlite IR-120, and extracted with n-BuOH saturated with water, and the extract was concentrated *in vacuo*. The residue was identified as **6** from the ^{1}H- and ^{13}C-NMR spectra.

Periplocoside N (**6**): Colorless needles, mp 236—238 °C, $[\alpha]_D$ −70.5 ° (c=0.18, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s, C-18), 1.00 (3H, s, C-19), 1.29 (3H, d, J=6.4 Hz, C-21), 1.33 (3H, d, J=6.1 Hz, can-6), 3.67 (1H, m, C-3), 3.73 (1H, q, J=6.3 Hz, C-20), 4.60 (1H, dd, J=9.7, 1.85 Hz, can-1), 5.34 (1H, br s, C-6).

Periplocoside L (**7**): Colorless needles, mp 238—240 °C and $[\alpha]_D$ −53.3 ° (c=0.06, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.75 (3H, s, C-18), 1.01 (3H, s, C-19), 1.19 (3H, d, J=6.3 Hz, C-21), 1.36 (3H, d, J=6.5 Hz, dig-6), 3.21 (1H, dd, J=7.8, 10.35 Hz, dig-3), 3.52 (3H, s, OMe), 3.84 (1H, q, J=6.3 Hz, C-20), 4.32 (1H, d, J=7.8 Hz, dig-1), 5.36 (1H, m, C-6).

S-20 (**10**): Colorless needles, mp 165—167 °C, $[\alpha]_D$ −65.2 ° (c=1.2, $CHCl_3$). EI-MS m/z: 300 [$M^+(C_{23}H_{36}O_3)$–AcOH]. ^{1}H-NMR ($CDCl_3$) δ: 0.77 (3H, s, C-18), 1.02 (3H, s, C-19), 1.15 (3H, d, J=6.8 Hz, C-21), 2.03 (3H, s, OAc), 3.73 (1H, dq, J=6.8, 2.0 Hz, C-20), 4.60 (1H, m, C-3), 5.37 (1H, m, C-6).

Acid Hydrolysis of Periplocosides D, E, L, M and N——Each sample (10 mg) was hydrolyzed with 0.05 N H_2SO_4 in 50% aqueous MeOH (3 ml) at 80 °C for 1 h. Each reaction mixture was diluted with water and the MeOH was evaporated off *in vacuo* at room temperature. The aqueous residue was extracted with $CHCl_3$ (×3) and the $CHCl_3$ layer was washed with water. After removal of the solvent, the residue was purified by means of silica gel column chromatography to give S-2A[1] or Δ^5-pregnene-3β,17α,20(S)-triol. The aqueous layer was neutralized with Amberlite IRA-94, and evaporated to dryness *in vacuo*. The residue showed the presence of cymarose (solv. 2, Rf=0.62; solv. 3, Rf=0.45), canarose (solv. 2, Rf=0.37; solv. 4, Rf=0.26) and digitalose (solv. 2, Rf=0.30; solv. 3, Rf=0.11) on silica gel TLC in comparison with authentic samples.

References and Notes

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