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Studies on Chemical Constituents of Antitumor Fraction from *Periploca sepium*. II. Structures of New Pregnane Glycosides, Periplocosides A, B and C

HIDEJI ITOKAWA,*,[a] JUNPING XU,[a] KOICHI TAKEYA,[a] KINZO WATANABE[a] and JUNZO SHOJI[b]

Tokyo College of Pharmacy,[a] Horinouchi 1432-1, Hachioji, Tokyo 192-03, Japan and School of Pharmaceutical Science, Showa University,[b] Hatanodai, Shinagawa-ku, Tokyo 142, Japan

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Three new pregnane glycosides, named periplocosides A, B and C, have been isolated from the antitumor fraction, which was obtained by subjecting the $CHCl_3$ extract of *Periploca sepium* to column chromatography over silica gel and eluting with $CHCl_3$–MeOH (10:1). Their structures were established by various nuclear magnetic resonance techniques and chemical evidence. The major constituents, periplocoside A, showed significant antitumor activity against Sarcoma 180 ascites in mice.

Keywords——*Periploca sepium*; Asclepiadaceae; antitumor substance; periplocoside A; 2D-NMR; ^{13}C–^{1}H long-range; pregnane glycoside; 3,7-dideoxy-4-*O*-methyl-α-D-gluco-2-heptulose

In the course of preliminary antitumor screening of crude drugs and collected plants,[1] it was found that the methanolic extract from the root bark of *Periploca sepium* (Asclepiadaceae) exhibited significant antitumor activity against Sarcoma 180 ascites in mice. In the preceding paper,[2] we reported the isolation of eight substances (S-1—S-8) from the antitumor fraction of the $CHCl_3$ extract of *P. sepium*, and the structures of their aglycones. This paper describes in detail the structural elucidation of the sugar moieties of three new pregnane glycosides S-2, S-7 and S-8, named periplocosides A, B and C, which were clarified with the aid of the two-dimensional nuclear magnetic resonance (2D-NMR) technique.[3]

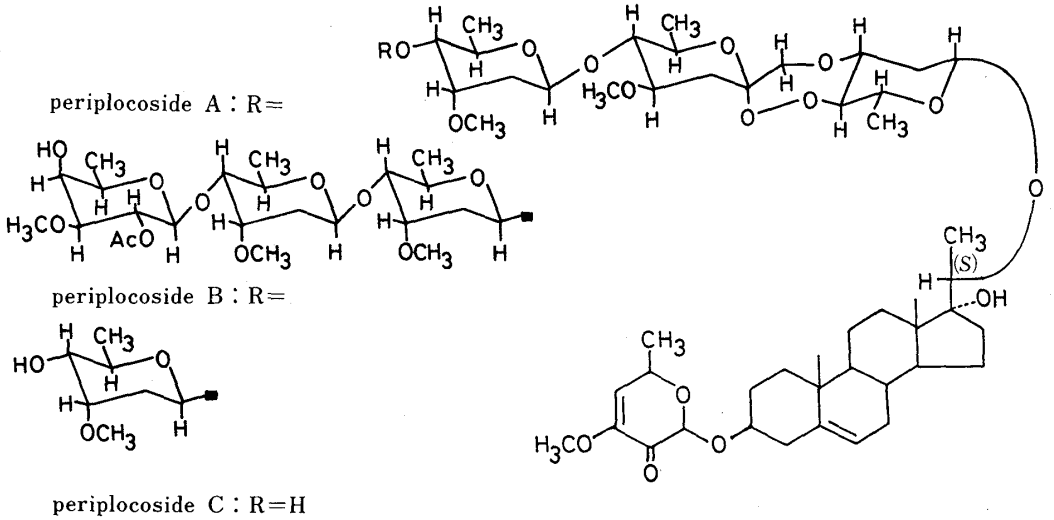

Fig. 1. Structures of Periplocosides A, B and C from *Periploca sepium*

Periplocoside A; colorless powder, mp 174—176 °C and $[\alpha]_D^{20}$ −1.2°, has the molecular formula $C_{72}H_{114}O_{27}\cdot 2H_2O$ from the elemental analysis (Calcd: C, 59.75; H, 8.12. Found: C, 59.61; H, 7.98), which was also supported by the fast atom bombardment mass spectrum (FAB-MS) *m/z*: 1433 ($M^+ + Na$), 1411 ($M^+ + H$). The hydrolysis of periplocoside A with 0.05 N H_2SO_4 in 50% aqueous MeOH gave S-2A and S-2A′, which were isolated by means of column chromatography on silica gel using $CHCl_3$–MeOH (96:4) and EtOAc as eluting solvents. Also, D-cymarose, D-canarose and D-digitalose from the hydrolyzate were identified by direct comparison with authentic samples on thin layer chromatography (TLC). The structure of S-2A was established as 3*β*-*O*-(4′,6′-dideoxy-3′-*O*-methyl-$\Delta^{3'}$-D-2′-hexosulosyl)-Δ^5-pregnene-3*β*,17*α*,20(*S*)-triol by various chemical and spectroscopic methods.[2] S-2A′, colorless needles (from EtOAc–MeOH), mp 170—171 °C, showed a molecular ion peak ($C_{17}H_{30}O_9$) at *m/z* 378 in the MS, and the presence of one acetyl group, three *O*-methyl groups, two secondary methyl groups and two *β*-anomeric protons in the ^{1}H-NMR spectrum, as can be seen from Table I. Upon the hydrolysis of S-2A′ with 0.1 N H_2SO_4, D-cymarose and D-digitalose were identified by TLC. Therefore, its structure was confirmed to be methyl 4-*O*-(2-*O*-acetyl-*β*-D-digitalopyranosyl)-*β*-D-cymaropyranoside.[4] The chemical shift of each carbon signal was assigned in comparison with the ^{13}C-NMR spectral data of methyl cymarose[5] and 2-*O*-acetyldigitalose[6] as shown in Table I. Consequently, S-2A′ was considered to be a part of the sugar chain of periplocoside A.

The ^{13}C-NMR spectrum of periplocoside A showed five doublet signals due to anomeric carbons of the sugar moiety at *δ* 98.59, 99.76 (×2), 100.81 and 102.58, and two unusual signals at *δ* 113.74 (s) and 86.40 (t) other than signals due to the aglycone.[2] Also, the ^{1}H-NMR spectrum exhibited six doublet methyl signals other than signals due to the aglycone. From the above first-order spectra, it was assumed that the sugar moiety of periplocoside A consisted of five 6-deoxyhexose residues and one 7-deoxyheptose. On the other hand, the C-20 carbon signal at *δ* 83.03 was shifted downfield (+10.68 ppm) in comparison with that of the aglycone, while no change of the C-17 chemical shifts between periplocoside A (*δ* 85.47) and the aglycone (*δ* 85.76) was recognized. Consequently, the sugar moiety of periplocoside A is linked to the C-20 hydroxyl group only. In the ^{1}H–^{1}H 2D-NMR spectrum of periplocoside A, the mutual relations from anomeric protons to C-6 methyl protons due to three D-cymarose, one D-canarose and one 2-*O*-acetyl-D-digitalose were fully elucidated as shown in Fig. 2. In addition, a similar sequence of protons from C-3 to C-7 methyl due to 3,7-dideoxyheptulose was seen, but no anomeric proton was observed in this heptulose. The ^{13}C–^{1}H 2D-NMR spectrum of periplocoside A readily led us to assign the ^{13}C chemical

TABLE I. ^{1}H and ^{13}C Chemical Shifts of S-2A′

	2-*O*-Acetyldigitalopyranose			Cymaropyranose		
1	4.37 (d)	*J*=8.0 Hz	102.47 (d)	4.61 (dd)	*J*=9.6, 2.2 Hz	98.98 (d)
2	5.07 (dd)	*J*=8.0, 9.8 Hz	70.88 (d)	1.51 (ddd)	*J*=13.6, 9.6, 2.5 Hz	35.44 (t)
				2.11 (ddd)	*J*=13.6, 6.2, 2.2 Hz	
3	3.25 (dd)	*J*=9.8, 3.8 Hz	81.56 (d)	3.78 (dd)	*J*=6.2, 3.0 Hz	76.37 (d)
4	3.85 (dd)	*J*=3.8, 1.5 Hz	68.00 (d)	3.16 (dd)	*J*=3.0, 9.5 Hz	83.16 (d)
5	3.57 (dq)	*J*=1.5, 6.5 Hz	70.34 (d)	3.87 (dq)	*J*=9.5, 6.3 Hz	68.21 (d)
6	1.35 (d)	*J*=6.5 Hz	16.39 (q)	1.18 (d)	*J*=6.3 Hz	17.88 (q)
OMe	3.44 (s)		57.34 (q)	3.42 (s)		58.31 (q)
OAc	2.05 (s, Me)		20.88 (q)	3.39 (s, OMe)		56.30 (q)
	(CO)		169.37 (s)			

The measurements were made on a Bruker AM400 spectrometer in $CDCl_3$ with TMS as an internal reference, and are expressed in terms of ppm.

TABLE II. ^{13}C Chemical Shifts of Periplocosides A, A′, B and C from *Periploca sepium*

		A	A′	B	C			A	A′	B	C
1	t	37.39	37.35	37.34	37.35	Cymarose (1)					
2	t	29.33	29.39	29.38	29.39	1	d	98.59	98.48	98.47	98.48
3	d	78.65	78.58	78.56	78.58	2	t	36.72	36.72	36.71	36.72
4	t	38.59	38.55	38.51	38.54	3	d	77.64	77.65	77.64	77.71
5	s	140.38	140.35	140.31	140.33	4	d	82.54	82.55	82.70	74.00
6	d	122.00	121.98	122.00	121.98	5	d	68.89	68.85	68.87	68.22
7	t	31.94	31.90	31.89	31.90	6	q	18.24	18.22	18.22	18.22
8	d	31.94	31.90	31.89	31.90	OMe	q	57.99	57.92	57.84	57.81
9	d	49.76	49.69	49.67	49.70	Cymarose (2)					
10	s	36.80	36.90	36.71	36.72	1	d	99.76	99.73	99.63	
11	t	20.60	20.56	20.55	20.57	2	t	35.61	35.55	36.70	
12	t	36.95	36.90	36.71	36.90	3	d	77.60	77.65	77.64	
13	s	45.39	45.34	45.33	45.34	4	d	82.54	82.49	73.65	
14	d	51.13	51.09	51.09	51.10	5	d	68.47	68.40	68.87	
15	t	23.48	23.45	23.46	23.46	6	q	18.24	18.22	18.22	
16	t	31.02	30.97	30.97	30.98	OMe	q	58.07	58.03	57.84	
17	s	85.47	85.45	85.47	85.45	Cymarose (3)					
18	q	14.15	14.12	14.13	14.12	1	d	99.76	99.73		
19	q	19.35	19.33	19.35	19.35	2	t	35.38	35.25		
20	d	83.03	83.05	83.13	83.10	3	d	77.35	77.64		
21	q	18.00	17.98	17.99	18.00	4	d	83.66	83.91		
1′	d	97.34	97.28	97.25	97.27	5	d	68.15	68.05		
2′	s	185.85	185.85	185.93	185.88	6	q	18.24	18.22		
3′	s	147.88	147.85	147.80	147.81	OMe	q	58.66	58.18		
4′	d	118.50	118.47	118.50	118.50	Digitalose					
5′	d	68.89	68.85	68.87	68.87	1	d	102.58	102.54		
6′	q	23.02	22.99	22.98	22.98	2	d	70.99	70.89		
OMe	q	54.97	54.95	54.97	54.96	3	d	81.62	80.05		
Canarose						4	d	68.09	68.40		
1	d	100.81	100.80	100.86	100.85	5	d	70.42	69.27		
2	t	38.42	38.40	38.39	38.40	6	q	16.50	16.53		
3	d	76.98	76.97	77.03	77.03	OMe	q	57.44	57.66		
4	d	79.24	79.19	79.21	79.23	OAc	s	169.40	169.34		
5	d	70.02	69.96	69.93	69.92		q	20.96	20.90		
6	q	17.02	17.04	17.08	17.07	OAc	s		170.85		
Heptulose							q		20.79		
1	t	86.40	86.37	86.38	86.38						
2	s	113.74	113.70	113.68	113.69						
3	t	36.75	36.72	36.71	36.72						
4	d	78.33	78.28	78.27	78.29						
5	d	82.65	82.55	82.70	82.57						
6	d	69.84	69.79	69.77	69.78						
7	q	18.00	17.98	17.99	18.00						
OMe	q	57.69	57.76	57.62	57.61						

Periplocoside A′ was obtained by acetylating periplocoside A with Ac_2O/pyridine. 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 2-*O*-acetyldigitalose may be reversed.

shifts due to the sugar moiety as shown in Table II, and the cross signal between the triplet carbon signal at δ 86.40 and each gem-proton at δ 4.74 and 5.14 (d, $J=7.5$ Hz, respectively) was also observed. Consequently, the presumed heptose was established to be 3,7-dideoxy-4-*O*-methyl-2-heptulose, whose structure was also supported by the long-range coupling between the carbon signal at δ 113.74 (s) and two gem-protons corresponding to the carbon

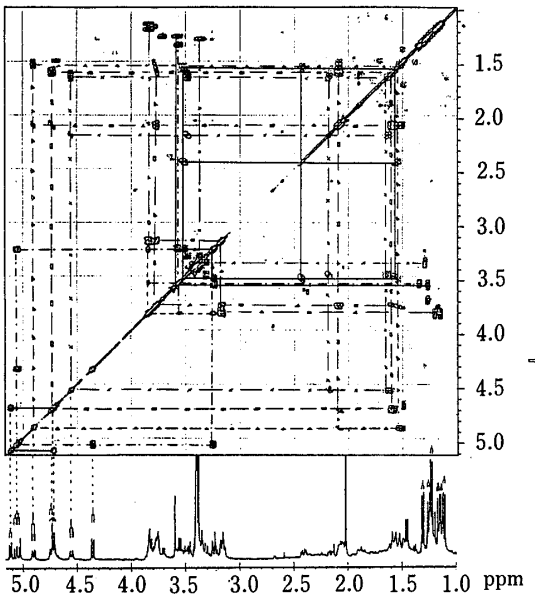

Fig. 2. ^{1}H–^{1}H 2D-NMR Spectrum of Periplocoside A in $CDCl_3$

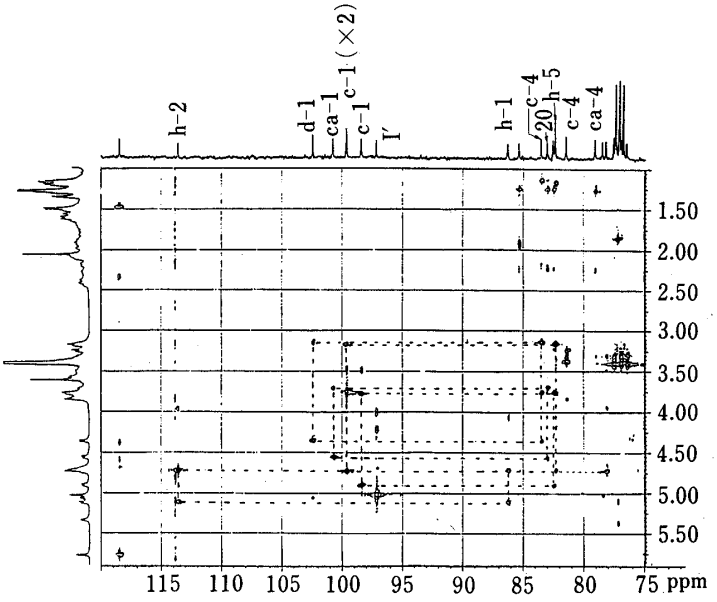

Fig. 3. ^{13}C–^{1}H Long-Range 2D-NMR Spectrum of Periplocoside A in $CDCl_3$

signal at δ 86.40 (t) in the ^{13}C–^{1}H long-range 2D-NMR spectrum (Fig. 3). The *J*-resolved 2D-NMR spectrum of the 3,7-dideoxy-4-*O*-methyl-2-heptulose moiety revealed each coupling constant, $J_{7,6}=6.5$ Hz, $J_{6,5}=9.5$ Hz, and $J_{5,4}=8.5$ Hz. Therefore, this heptose was confirmed to be 3,7-dideoxy-4-*O*-methyl-D-gluco-2-heptulopyranose.[7] The *J*-resolved spectrum also indicated $J_{6,5}=6.0$ Hz, $J_{5,4}=9.0$ Hz, $J_{4,3}=7.5$ Hz and $J_{2,1}=9.5$, 1.5 Hz due to β-D-canaropyranose, $J_{6,5}=6.5$ Hz, $J_{5,4}=9.5$ Hz, $J_{4,3}=3.0$ Hz and $J_{2,1}=10$, 1.5 Hz due to β-D-cymaropyranose, and $J_{6,5}=6.5$ Hz, $J_{5,4}=1.5$ Hz, $J_{4,3}=3.8$ Hz, $J_{3,2}=10$ Hz and $J_{2,1}=8.0$ Hz due to 2-*O*-acetyl-β-D-digitalopyranose. When the ^{13}C–^{1}H long-range 2D-NMR was measured in order to determine the sequence of the six sugars in the sugar moiety of periplocoside A, it showed the cross signals due to five ^{3}J coupling between each anomeric carbon or proton and each other proton or carbon, namely C-1 of 2-*O*-acetyldigitalose and C-4 of cymarose (3); C-1 of cymarose (3) and C-4 of cymarose (2); C-1 of cymarose (2) and C-4 of cymarose (1); C-1 of cymarose (1) and C-5 of 3,7-dideoxy-4-*O*-methylgluco-2-heptulose; C-1 of the heptulose and C-3 of canarose; C-1 of canarose and C-20 of the aglycone. The ^{13}C–^{1}H long-range coupling between C-2 of the heptulose and C-4 of canarose could not be observed, but the above sugar-junction seemed reasonable, because only the 4-hydroxyl group in the terminal sugar of the sugar chain was acetylated with Ac_2O/pyridine at room temperature, the C-4 carbon signal of canarose was shifted downfield about 2.0 ppm, and its positive color reaction for an peroxide[8] was observed. Since the stabler conformation of D-gluco-2-heptulose has no *cis* relation of axial substituents, it was indicated that the configuration was solely α form, in accord with the observation by Perlin *et al.*[9] Therefore, 3,7-dideoxy-4-*O*-methyl-D-gluco-2-heptulopyranose of the sugar moiety was also suggested to be α form. Thus, periplocoside A 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*-(2-*O*-acetyl-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 B and C were obtained as minor constituents of this plant. Their ^{1}H-and ^{13}C-NMR spectra were similar to those of periplocoside A except for signals of the sugar moieties. Upon acid hydrolysis of periplocosides B and C, cymarose and canarose, re-

spectively, were identified by direct comparison with authentic samples on TLC. Therefore, periplocosides B and C were assumed to be structurally related to periplocoside A.

Periplocoside B, colorless powder, mp 136—138 °C and $[\alpha]_D^{20}$ +1.9°, showed three anomeric carbon signals (δ 98.47, 99.63 and 100.86) and two characteristic signals (δ 113.68 (s) and 86.38 (t)) in the ^{13}C-NMR spectrum. The signals were concluded to be due to β-D-cymarose (×2), β-D-canarose and 3,7-dideoxy-4-*O*-methyl-α-D-heptulose by comparison with those of periplocoside A, and the structure of periplocoside B was established 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-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 C, colorless powder, mp 180—182 °C and $[\alpha]_D^{20}$ −8.4°, exhibited two anomeric carbon signals (δ 98.48 and 100.85) and two characteristic signals (δ 113.69 (s) and 86.38 (t)) in the ^{13}C-NMR spectrum, which indicated the presence of β-D-cymarose, β-D-canarose and 3,7-dideoxy-4-*O*-methyl-α-D-gluco-2-heptulose in the sugar moiety. The structure of periplocoside C was elucidated as 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-cymaropyranosyl(1→5)-3,7-dideoxy-*O*-methyl-α-D-gluco-2-heptulopyranosyl(2→4)-dioxy-(1→3)-β-D-canaropyranoside) from a comparison of the ^{13}C signals with those of periplocosides A and B.

The major component, periplocoside A, showed significant antitumor activity against Sarcoma 180 ascites in mice (20 mg/kg/d dose for 5 consecutive days, GR (growth ratio = T/C × 100): 20% (++); 10 mg/kg/d, GR: 53% (+)) and other antitumor activity studies are in progress.

Experimental

All melting points were recorded on a Yanagimoto micro melting point apparatus and are uncorrected. The NMR spectra were taken on a Bruker AM400 instrument at 400 MHz for ^{1}H 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). FAB-MS were measured on a JEOL JMS DX-303 spectrometer and electron ionization (EI-MS) on a Hitachi M-80 instrument.

The following solvent systems were used for 0.25 mm Kieselgel F_{254} (Merck) TLC: 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); 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. The extraction and isolation of periplocoside A, B and C from *P. sepium* have been described in the previous paper.[2)]

Periplocoside A——Colorless powder. mp 174—176 °C $[\alpha]_D^{20}$ −1.2° (*c* = 1.4, $CHCl_3$). $C_{72}H_{114}O_{27}$. FAB-MS *m/z*: 1433 $[M+Na]^+$, 1411 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s, C-18), 1.00 (3H, s, C-19), 1.17, 1.19, 1.21 (3H, d, *J* = 6.5 Hz, cym-6, respectively), 1.29 (6H, 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), 1.51 (3H, d, *J* = 6.8 Hz, C-6′), 3.41, 3.42, 3.43, 3.44, 3.45 (3H, s, OMe, respectively), 3.57 (3H, s, C-3′OMe), 3.67 (1H, m, C-3), 3.70 (1H, q, *J* = 6.5 Hz, C-20), 4.38 (1H, d, *J* = 8.0 Hz, dig-1), 4.58 (1H, dd, *J* = 9.5, 1.5 Hz, can-1), 4.70 (1H, ddq, *J* = 6.8, 3.0, 0.5 Hz, C-5′), 4.74 (1H, d, *J* = 7.5 Hz, hep-1), 4.76 (1H, dd, *J* = 10, 1.5 Hz, cym-1 ×2), 4.92 (1H, dd, *J* = 10, 1.5 Hz, cym-1), 4.94 (1H, d, *J* = 0.5 Hz, C-1′), 5.09 (1H, dd, *J* = 10, 8.0 Hz, dig-2), 5.14 (1H, d, *J* = 7.5 Hz, hep-1), 5.35 (1H, br s, C-6), 5.77 (1H, d, *J* = 3.0 Hz, C-4′). The acetylation of periplocoside A was carried out in the usual way, and the acetate was obtained as a colorless powder, mp 154—156 °C. $[\alpha]_D^{25}$ −1.0° (*c* = 0.2, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s, C-18), 1.00 (3H, s, C-19), 1.17, 1.19, 1.21 (3H, d, *J* = 6.5 Hz, cym-6, respectively), 1.22 (3H, *J* = 6.5 Hz, dig-6), 1.29 (3H, d, *J* = 6.2 Hz, C-21), 1.29 (3H, d, *J* = 6.5 Hz, hep-7), 1.31 (3H, d, *J* = 6.0 Hz, can-6), 1.51 (3H, d, *J* = 6.8 Hz, C-6′), 2.06, 2.15 (3H, s, -OCOMe, respectively), 3.32, 3.42, 3.43, 3.44, 3.45 (3H, s, OMe, respectively), 3.63 (3H, s, C-3′OMe), 4.42 (1H, d, *J* = 8.0 Hz, dig-1), 4.57 (1H, dd, *J* = 9.5, 1.5 Hz, can-1), 4.74 (1H, d, *J* = 7.5 Hz, hep-1), 4.73, 4.76, 4.92 (1H, dd, *J* = 10, 1.5 Hz, cym-1, respectively), 5.05 (1H, s, C-1′), 5.11 (1H, dd, *J* = 10.1, 8.0 Hz, dig-2), 5.13 (1H, d, *J* = 7.5 Hz, hep-1), 5.31 (1H, dd, *J* = 3.3, 1.2 Hz, dig-4), 5.36 (1H, br s, C-6), 5.58 (1H, d, *J* = 3.0 Hz, C-4′).

Periplocoside B——Colorless powder. mp 136—138 °C. $[\alpha]_D^{20}$ +1.9° (*c* = 0.2, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.73 (3H, s, C-18), 1.00 (3H, s, C-19), 1.20, 1.23 (3H, d, *J* = 6.5 Hz, cym-6, respectively), 1.29 (6H, d, *J* = 6.5 Hz, C-21 and hep-7), 1.31 (3H, d, *J* = 6.0 Hz, can-6), 3.41, 3.43, 3.44 (3H, s, OMe, respectively), 3.63 (3H, s, C-3′OMe), 3.67 (1H, m, C-3), 3.70 (1H, q, *J* = 6.5 Hz, C-20), 4.58 (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* = 7.3 Hz, hep-1), 4.76, 4.92 (1H, d, *J* = 10, 1.5 Hz, cym-1, respectively), 5.05 (1H, s, C-1′), 5.14 (1H, d, *J* = 7.3 Hz,

hep-1), 5.36 (1H, br s, C-6), 5.78 (1H, d, $J=3.0$ Hz, C-4′).

Periplocoside C——Colorless powder, mp 180—182 °C. $[\alpha]_D^{20}$ −8.4° ($c=0.3$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.72 (3H, s, C-18), 1.00 (3H, s, C-19), 1.22 (3H, d, $J=6.5$ Hz, cym-6), 1.29 (6H, d, $J=6.5$ Hz, C-21 and hep-7), 1.31 (3H, d, $J=6.0$ Hz, can-6), 1.51 (3H, d, $J=6.8$ Hz, C-6′), 3.41, 3.44 (3H, s, OMe, respectively), 3.63 (3H, s, C-3′OMe), 3.67 (1H, m, C-3), 3.70 (1H, q, $J=6.5$ Hz, C-20), 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, $J=7.5$ Hz, hep-1), 4.76 (1H, dd, $J=10$, 1.5 Hz, cym-1), 5.04 (1H, s, C-1′), 5.13 (1H, d, $J=7.5$ Hz, hep-1), 5.36 (1H, br s, C-6), 5.78 (1H, d, $J=3.0$ Hz, C-4′).

S-2A′——Colorless needles (from EtOAc–MeOH). mp 170—171 °C. $[\alpha]_D^{20}$ −51.8° ($c=0.25$, MeOH). EI-MS m/z: 378 (M^+).

Acid Hydrolysis of Periplocosides A, B and C and S-2A′——Periplocoside A (25 mg) was hydrolyzed with 0.05 N H_2SO_4 in 50% aqueous MeOH (6 ml) at 80 °C for 1 h. The reaction mixture was diluted with water and concentrated *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 with $CHCl_3$–MeOH (96 : 4) to give S-2A (5 mg) and S-2A′ (4 mg). The aqueous layer was neutralized with Amberlite IRA-94, and evaporated to dryness *in vacuo*. The residue showed the presence of D-cymarose (solv.2, $Rf=0.62$; solv.3, $Rf=0.45$), D-canarose (solv.2, $Rf=0.37$; solv.4, $Rf=0.26$) and D-digitalose (solv.2, $Rf=0.30$; solv.3, $Rf=0.11$) on silica gel TLC in comparison with authentic samples. A sample (8 mg) of each periplocosides B and C (0.05 N) and S-2A′ (0.1 N) was hydrolyzed under the same conditions as described above. The residue obtained in a similar manner as above contained D-cymarose and D-canarose in the cases of periplocosides B and C, and D-digitalose and D-cymarose in the case of S-2A′, as determined by TLC.

Assay of Activity against Sarcoma 180 Ascites——ICR male mice, 5 weeks old, supplied by Clea Japan Co., Ltd., were used in groups of 6 animals. Sarcoma 180 ascites were implanted i.p. at 1×10^6 cells/body. Administration of a test drug was started at one day after the implantation and continued for 5 d by the i.p. route. The effectiveness was evaluated by means of the total packed cell volume method,[10] growth ratio (GR) % = (packed cell volume (PCV) of test group/PCV of control group) × 100; GR = 0—10% (+++), 11—40% (++), 41—65% (+) and over 66% (−).

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