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**Studies on Chemical Constituents of Antitumor Fraction from
Periploca sepium. V. Structures of New Pregnane
Glycosides, Periplocosides J, K, F and O**

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Four new pregnane glycosides, named periplocosides J, K, F and O, have been isolated from the antitumor fraction of *Periploca sepium* (Asclepiadaceae). Their structures were established by various nuclear magnetic resonance techniques and chemical evidence.

Keywords—*Periploca sepium*; Asclepiadaceae; pregnane glycoside; 3,7-dideoxy-4-*O*-methyl-D-glucosyl-2-heptulose; 2D-NMR; periplocoside

In the preceding papers of this series,¹⁻⁴⁾ we described eight new pregnane glycosides, named periplocosides A, B, C, D, E, L, M and N, which were isolated from the antitumor fraction of the CHCl_3 extract of *Periploca sepium* (Asclepiadaceae). These structures were clarified with the aid of the two-dimensional nuclear magnetic resonance (2D-NMR) technique and partial acid hydrolysis. The present paper deals with the isolation and structural elucidations of four new pregnane glycosides, S-16, 17, 18 and 19, named periplocosides J, K, F and O, respectively.

Periplocoside J (**1**), colorless powder, mp 178—181 °C, $[\alpha]_{\text{D}}^{20} + 24.13^\circ$ ($c = 0.12$, MeOH) gave the molecular formula $\text{C}_{61}\text{H}_{100}\text{O}_{23}$, based on its $[\text{M} + \text{Na} + \text{H}]^+$ peak at m/z 1224 and $[\text{M} + \text{K} + \text{H}]^+$ peak at m/z 1240 in the secondary ion mass spectrum (SIMS). The proton and carbon-13 nuclear magnetic resonance (^1H - and ^{13}C -NMR) (Table I) spectral data showed five anomeric signals [δ 4.28 (d), 4.54, 4.57, 4.75, 4.99 (dd, respectively)], seven doublet methyl signals due to 6-deoxysugar and C-21 of the aglycone (δ 1.23, 1.25, 1.28, 1.29, 1.31, 1.32, 1.35), three methoxyl signals (δ 3.44, 3.46, 3.51) and two characteristic signals due to 3,7-dideoxy-4-*O*-methyl-D-glucosyl-2-heptulose²⁾ [δ 4.72, 5.13 (each d, $J = 7.5$ Hz) and 86.38 (t), 113.70 (s)], and showed a glycosylation shift at C-20 of the aglycone (+10.7 ppm). The hydrolysis of **1** with 0.05 N H_2SO_4 in 50% aqueous MeOH gave Δ^5 -pregnene 3 β ,17 α ,20(*S*)-triol (**5**)¹⁾ as the aglycone and a disaccharide (**6**) which was confirmed to be methyl β -D-digitalopyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranoside.^{2,5)} In addition, digitalose, digitoxose, canarose and cymarose from the hydrolyzate were identified by direct thin-layer chromatographic (TLC) comparison with authentic samples. From the ^1H - ^1H 2D-NMR spectrum, the mutual relations from the anomeric protons to C-6 methyl protons due to four 2,6-dideoxyhexoses and one digitalose, and from the C-3 proton to the C-7 methyl proton due to 3,7-dideoxy-4-*O*-methyl-D-glucosyl-2-heptulose were fully elucidated. In order to confirm each coupling constant due to those mutual relations of the sugar moiety, the *J*-resolved 2D-NMR spectrum was measured to reveal $J_{6,5} = 6.5$ Hz, $J_{5,4} = 1.5$ Hz, $J_{4,3} = 3.5$ Hz, $J_{3,2} = 9.4$ Hz, $J_{2,1} = 7.8$ Hz due to one β -D-digitalose, $J_{6,5} = 6.2$ Hz, $J_{5,4} = 9.5$ Hz, $J_{4,3} = 3.5$ Hz, $J_{2,1} = 9.8$, 1.8 Hz due to one β -D-cymarose, $J_{6,5} = 6.0$ Hz, $J_{5,4} = 9.5$ Hz, $J_{4,3} = 8.6$ Hz, $J_{2,1} = 9.6$, 1.7 Hz due to two β -D-canarose, $J_{6,5} = 6.1$ Hz, $J_{5,4} = 9.8$ Hz, $J_{4,3} = 3.0$ Hz, $J_{2,1} = 9.8$, 1.8 Hz due to one β -D-digitoxose and $J_{7,6} = 6.5$ Hz, $J_{6,5} = 9.8$ Hz, $J_{5,4} = 8.2$ Hz, $J_{1a,1b} = 7.5$ Hz due to one 3,7-dideoxy-4-*O*-methyl-D-glucosyl-

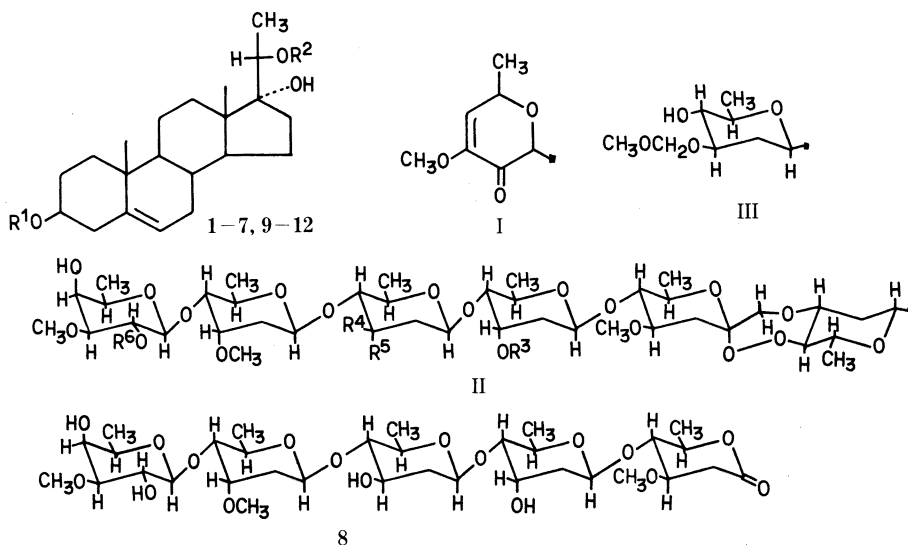
2-heptulose.

On the partial hydrolysis of **1** with 0.001 N H₂SO₄ in MeOH at room temperature, the hydrolyzate was found to contain **5**, periplocoside N [Δ^5 -pregnene 3 β ,17 α ,20(*S*)-triol 20- β -D-canaropyranoside] (**7**)^{3,6)} and **6** by TLC comparison with authentic samples, as well as the product (**8**). Therefore, it was considered that canarose was the initial sugar in the sugar chain. The ¹H-NMR, ¹³C-NMR (Table I) and infrared (IR) spectra of **8** showed the presence of five doublet methyl protons (δ 1.22, 1.24, 1.27, 1.34, 1.44), three methoxyl groups (δ 3.39, 3.42, 3.46), one δ -lactone (δ 168.09 and 1750 cm⁻¹) and four anomeric signals (δ 4.39, 4.54, 4.75, 4.99). By comparison of its ¹H-, ¹³C- and ¹H-¹H 2D-NMR spectra with those of **1** and D-

TABLE I. ¹³C Chemical Shifts of Periplocosides J (**1**), K (**2**), F (**3**) and O (**4**)

		1	2	3	4		
		1	2	3	4	1	2
1	t	37.25	37.37	37.29	37.38	Heptulose	
2	t	31.87	29.41	31.89	29.41	1	t
3	d	71.74	78.62	71.76	78.64	2	s
4	t	42.30	38.56	42.34	38.52	3	t
5	s	140.71	140.35	140.75	140.37	4	d
6	d	121.63	122.01	121.63	121.96	5	d
7	t	31.94	31.92	31.98	31.94	6	d
8	d	31.67	31.92	31.71	31.94	7	q
9	d	49.64	49.72	49.69	49.74	OMe	q
10	s	36.66	36.74	36.77	36.74	Digitoxose or cymarose	
11	t	20.58	20.58	20.61	20.59	1	d
12	t	36.90	36.89	36.93	38.44	2	t
13	s	45.34	45.37	45.37	45.38	3	d
14	d	51.08	51.12	51.11	51.14	4	d
15	t	23.48	23.47	23.50	23.49	5	d
16	t	30.97	31.00	31.00	31.02	6	q
17	s	85.45	85.47	85.46	85.48	OMe	q
18	q	14.14	14.14	14.15	14.15	Canarose or cymarose	
19	q	19.40	19.35	19.40	19.36	1	d
20	d	85.45	85.47	85.46	82.86	2	t
21	q	17.99	18.01	18.00	18.07	3	d
1'	d		97.30		97.32	4	d
2'	s		185.87		185.91	5	d
3'	s		147.85		147.88	6	q
4'	d		118.49		118.49	OMe	
5'	d		68.89		68.89	Cymarose	
6'	q		23.01		23.03	1	d
OMe	q		54.98		54.97	2	t
Canarose						3	d
1	d	100.82	100.82	100.82	100.80	4	d
2	t	38.40	38.42	38.41	37.76	5	d
3	d	77.03	77.02	77.03	81.45	6	q
4	d	79.19	79.22	79.22	75.35	OMe	q
5	d	69.95	69.96	70.00	71.79	Digitalose	
6	q	17.06	17.06	17.06	17.01	1	d
					96.98 t	2	d
					(-OCH ₂ O-)	3	d
					55.74 q	4	d
					(OMe)	5	d
						6	q
						OMe	q

The measurements were made on a Bruker AM400 instrument in CDCl₃ 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.



- 1: $R^1 = H$, $R^2 = II$, $R^3 = H$, $R^4 = OH$, $R^5 = H$, $R^6 = H$
- 2: $R^1 = I$, $R^2 = II$, $R^3 = H$, $R^4 = OH$, $R^5 = H$, $R^6 = H$
- 3: $R^1 = H$, $R^2 = II$, $R^3 = CH_3$, $R^4 = H$, $R^5 = OCH_3$, $R^6 = H$
- 4: $R^1 = I$, $R^2 = III$
- 5: $R^1 = H$, $R^2 = H$
- 6: methyl β -D-digitalopyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranoside
- 7: $R^1 = H$, $R^2 = \beta$ -D-canarose
- 9: $R^1 = I$, $R^2 = II$, $R^3 = CH_3$, $R^4 = H$, $R^5 = OCH_3$, $R^6 = Ac$
- 10: $R^1 = I$, $R^2 = H$
- 11: $R^1 = H$, $R^2 = II$, $R^3 = CH_3$, $R^4 = H$, $R^5 = OCH_3$, $R^6 = Ac$
- 12: $R^1 = I$, $R^2 = \beta$ -D-canarose

Chart 1

oleandronic δ -lactone,⁷⁾ it has become apparent that **8** consists of β -D-digitalose, β -D-digitoxose, β -D-canarose, β -D-cymarose and D-oleandronic δ -lactone. The sequence of each sugar in **8** was deduced to be as shown in Chart 1, because cross signals between the C-1 positional proton (H-1) of cymarose and H-4 of canarose, and H-1 of canarose and H-4 of digitoxose were observed in the 1H - 1H two-dimensional nuclear Overhauser enhancement and exchange spectroscopy (2D-NOESY) spectrum. Consequently, the structure of **8** was determined to be β -D-digitalopyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranosyl-(1 $\rightarrow$ 4)-D-oleandro-1,5-lactone.⁸⁾

In the ^{13}C -NMR spectrum of **1**, the chemical shifts of signals due to 3,7-dideoxy-4-O-methyl-D-*gluco*-2-heptulose and initial canarose moieties in the sugar chain were coincident with those of periplocoside A (**9**).^{2,9)} In particular, the signals at C-3 and C-4 of the initial canarose were also shifted downfield about 5.4 and 1.8 ppm, respectively, in comparison with those of periplocoside N (**7**).³⁾ In addition, a positive color reaction for peroxide was observed.¹¹⁾ Consequently, we considered that the hydroxyl groups at C-1 and C-2 of the heptose in the sugar chain of **1** are respectively combined with C-3 and C-4 of the canarose, in a peroxide form at only the latter bond, and the hydrolyzate **8** results from fission of this O-O bond.³⁾ From the above results, the structure of periplocoside J (**1**) was established as Δ^5 -pregnene 3 β ,17 α ,20(*S*)-triol 20-O- β -D-digitalopyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranosyl-(1 $\rightarrow$ 5)-3,7-dideoxy-4-O-methyl- α -D-*gluco*-2-heptulopyranosyl-(2 $\rightarrow$ 4)-dioxy-(1 $\rightarrow$ 3)- β -D-canaropyranoside.

Periplocoside K (**2**), colorless powder, mp 208–212 °C, $[\alpha]_D^{20} -4.76^\circ$ ($c=0.08$, MeOH)

gave the molecular formula $C_{68}H_{108}O_{26}$ from its $[M+Na]^+$ at m/z 1363 and $[M+K]^+$ at m/z 1379 in the SIMS. Compound **2** was hydrolyzed with 0.05 N H_2SO_4 in 50% aqueous MeOH to afford Δ^5 -pregnene-3 β ,17 α ,20(*S*)-triol 3-*O*-(4',6'-dideoxy-3'-*O*-methyl- Δ^3 -D-2'-hexosulopyranoside) (**10**),¹⁾ **6**, cymarose, canarose, digitalos and digitoxose, which were identified by direct TLC comparison with authentic samples. The ^{13}C -NMR spectrum of **2** showed six anomeric signals due to one cymarose, one digitoxose, one digitalose, two canarose, one 4,6-dideoxy-3-*O*-methyl- Δ^3 -D-2-hexosulose and two characteristic signals [δ 113.72 (s), 86.39 (t)] due to 3,7-dideoxy-4-*O*-methyl-D-*gluco*-2-heptulose other than signals due to **5**, and showed a glycosylation shift at C-20 of the aglycone. Also, it gave a positive color reaction for peroxide,¹¹⁾ and the 1H - and ^{13}C -NMR spectra of **2** were compatible with those of **1** except for the signals due to the 4',6'-dideoxy-3'-*O*-methyl- Δ^3 -D-2'-hexosulose moiety. Therefore, **2** was deduced to be Δ^5 -pregnene-3 β ,17 α ,20(*S*)-triol 3-*O*-(4',6'-dideoxy-3'-*O*-methyl- Δ^3 -D-2'-hexosulose) 20-*O*-(β -D-digitalopyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-canaropyranosyl-(1 $\rightarrow$ 4)- β -D-digitoxopyranosyl-(1 $\rightarrow$ 5)-3,7-dideoxy-4-*O*-methyl- α -D-*gluco*-2-heptulopyranosyl-(2 $\rightarrow$ 4)-dioxy-(1 $\rightarrow$ 3)- β -D-canaropyranoside).

Periplocoside F (**3**), colorless powder, mp 195–198 °C, $[\alpha]_D^{20} + 8.1^\circ$ ($c=0.07$, MeOH) was hydrolyzed in the same way as for **1** to furnish **5**, canarose, cymarose and digitalose which were identified by direct TLC comparison with authentic samples. The 1H - and ^{13}C -NMR signals of **3** were similar to those of periplocoside E (**11**)³⁾ except for the signals due to the acetyl group at C-2 of digitalose in the terminal sugar of the sugar chain. Also, it gave a positive color reaction for peroxide,¹¹⁾ and a glycosylation shift in the ^{13}C -NMR spectrum of **3** was observed at C-20 of its aglycone. Compound **3** was acetylated with Ac_2O /pyridine at room temperature to afford its triacetate, which was identical with an acetate prepared from periplocoside E (**11**)³⁾ in the same manner. Accordingly, **3** was concluded to be Δ^5 -pregnene-3 β ,17 α ,20(*S*)-triol 20-*O*-(β -D-digitalopyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranosyl-(1 $\rightarrow$ 5)-3,7-dideoxy-4-*O*-methyl- α -D-*gluco*-2-heptulopyranosyl-(2 $\rightarrow$ 4)-dioxy-(1 $\rightarrow$ 3)- β -D-canaropyranoside).

Periplocoside O (**4**), colorless powder, mp 103–106 °C, $[\alpha]_D^{20} - 84.0^\circ$ ($c=0.05$, MeOH) gave **5** in acid hydrolysis as described above. Compound **4** showed two anomeric signals due to a canarose analog (δ 4.58 and 100.80) and 4,6-dideoxy-3-*O*-methyl- Δ^3 -2-hexosulose (δ 5.05 and 97.32) in the 1H - and ^{13}C -NMR spectra, and glycosylation shifts at C-3 and C-20 of the aglycone were recognized. In addition, its NMR spectra exhibited characteristic signals due to a dioxymethylene group (δ 4.72 and 96.98) and one more methoxyl group (δ 3.43 and 55.73) in comparison with those of periplocoside M (**12**),³⁾ and the carbon signals corresponding to C-3 and C-4 of the canarose were shifted +9.8 and –2.1 ppm, respectively. Therefore, it was presumed that a methoxymethyl group was linked to the C-3 hydroxyl group of the canarose. This unique canarose analog might be produced by peroxide bond cleavage between canarose and heptulose in the sugar chains of periplocosides.³⁾ Consequently, **4** was concluded to be Δ^5 -pregnene-3 β ,17 α ,20(*S*)-triol 3-*O*-(4',6'-dideoxy-3-*O*-methyl- Δ^3 -D-2'-hexosulose) 20-*O*-(3-*O*-methoxymethyl- β -D-canaropyranoside).

Experimental

All melting points were recorded on a Yanagimoto MP-3 micro melting point apparatus and are uncorrected. The NMR spectra were taken on a Bruker AM 400 instrument at 400 MHz (1H) and 100.6 MHz (^{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 JEOL JMS DX-303 spectrometer.

The following solvent systems were used for TLC on 0.25 mm Kieselgel F₂₅₄ (Merck) 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); solvent 4, $CHCl_3$ -MeOH (2:1) and solvent 5, EtOAc. Each spot on a TLC plate was detected by spraying 10% H_2SO_4 and heating the plate.

Isolation of S-16, 17, 18 and 19—As reported in the previous paper,¹⁻³⁾ the CM-1 fraction (20 g) was subjected to chromatography on silica gel and eluted with CHCl_3 -MeOH (9:1) and (5:1), respectively. The obtained CHCl_3 -MeOH (5:1) fraction was submitted to high performance liquid chromatography (HPLC) on RP-18 column and eluted with MeOH- H_2O (8:2) and (7:3) to furnish S-16 (25 mg), S-17 (10 mg), S-18 (12 mg) and S-19 (7 mg), named periplocosides J, K, F and O, respectively.

Periplocoside J (1)—Colorless powder, mp 178–181 °C, $[\alpha]_D^{20} +24.13^\circ$ ($c=0.12$, MeOH). SIMS m/z : 1224 $[\text{M}(\text{C}_{61}\text{H}_{100}\text{O}_{23}) + \text{Na} + \text{H}]^+$, 1240 $[\text{M} + \text{K} + \text{H}]^+$. $^1\text{H-NMR}$ (CDCl_3) δ : 0.73 (3H, s, 18- CH_3), 0.97 (3H, s, 19- CH_3), 1.23 (3H, d, $J=6.2$ Hz, cym-6), 1.25 (3H, d, $J=6.1$ Hz, digt-6), 1.28 (3H, d, $J=6.3$ Hz, 21- CH_3), 1.29 (3H, d, $J=6.5$ Hz, hep-7), 1.31 (3H, d, $J=6.0$ Hz, can'-6), 1.32 (3H, d, $J=6.0$ Hz, can-6), 1.35 (3H, d, $J=6.5$ Hz, dig-6), 2.96 (1H, dd, $J=9.5$, 8.6 Hz, can'-4), 3.44, 3.46, 3.51 (each 3H, s, OMe), 3.68 (1H, dd, $J=9.4$, 7.8 Hz, dig-2), 4.28 (1H, d, $J=7.8$ Hz, dig-1), 4.54 (1H, dd, $J=9.6$, 1.65 Hz, can-1), 4.57 (1H, dd, $J=9.6$, 1.65 Hz, can'-1), 4.72 (1H, d, $J=7.5$ Hz, hep-1a), 4.75 (1H, dd, $J=9.8$, 1.8 Hz, cym-1), 4.99 (1H, dd, $J=9.7$, 1.9 Hz, digt-1), 5.13 (1H, d, $J=7.5$ Hz, hep-1b), 5.34 (1H, m, 6-H).

Partial Hydrolysis of Periplocoside J (1)—A solution of 1 (20 mg) in 0.001 N H_2SO_4 -MeOH (2 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 showed the presence of Δ^5 -pregnene-3 β ,17 α ,20(S)-triol (5) (solv. 3, $R_f=0.51$, solv. 5, $R_f=0.50$), periplocoside N (7) (solv. 3, $R_f=0.34$, solv. 5, $R_f=0.23$), and methyl β -D-digitalopyranosyl-(1 $\rightarrow$ 4)- β -D-cymaropyranoside (6) (solv. 3, $R_f=0.47$, solv. 5, $R_f=0.16$) on TLC, and gave 8 (1.5 mg), which was purified by means of silica gel column chromatography using EtOAc as the eluent.

Hydrolyzate 8—Colorless powder, mp 253–256 °C, $[\alpha]_D^{20} +28.5^\circ$ ($c=0.05$, CHCl_3). $^1\text{H-NMR}$ (CDCl_3) δ : 1.22 (3H, d, $J=6.2$ Hz, cym-6), 1.24 (3H, d, $J=6.1$ Hz, digt-6), 1.27 (3H, d, $J=6.0$ Hz, can-6), 1.34 (3H, d, $J=6.5$ Hz, dig-6), 1.44 (3H, d, $J=6.45$ Hz, lactone-6), 2.72 (2H, t, $J=4.0$ Hz, lactone-2), 2.95 (1H, dd, $J=9.6$, 8.5 Hz, can-4), 3.39, 3.42, 3.46 (each 3H, s, OMe), 4.39 (1H, d, $J=6.5$ Hz, dig-1), 4.54 (1H, dd, $J=9.6$, 1.7 Hz, can-1), 4.75 (1H, dd, $J=9.8$, 1.8 Hz, cym-1), 4.99 (1H, dd, $J=9.7$, 1.8 Hz, digt-1).

Acetylation of 8—The acetylation of 8 was carried out in the usual way with Ac_2O /pyridine to give the acetate as a colorless powder. $^1\text{H-NMR}$ (CDCl_3) δ : 1.16 (3H, d, $J=6.2$ Hz, cym-6), 1.21 (3H, d, $J=6.1$ Hz, digt-6), 1.24 (3H, d, $J=6.0$ Hz, can-6), 1.26 (3H, d, $J=6.5$ Hz, dig-6), 1.43 (3H, d, $J=6.4$ Hz, lactone-6), 2.05, 2.06, 2.10, 2.15 (each 3H, s, OAc), 2.71 (2H, br d, lactone-2), 3.33, 3.39, 3.43 (each 3H, s, OMe), 4.41 (1H, d, $J=7.8$ Hz, dig-1), 4.55 (1H, dd, $J=9.6$, 1.7 Hz, can-1), 4.76 (1H, dd, $J=9.8$, 1.8 Hz, cym-1), 4.85 (1H, dd, $J=9.7$, 1.8 Hz, digt-1), 5.09 (1H, dd, $J=9.8$, 8.0 Hz, dig-2), 5.31 (1H, br d, dig-4).

Periplocoside K (2)—Colorless powder, mp 208–212 °C, $[\alpha]_D^{20} -4.76^\circ$ ($c=0.08$, MeOH). SIMS m/z : 1363 $[\text{M}(\text{C}_{68}\text{H}_{108}\text{O}_{26}) + \text{Na}]^+$, 1379 $[\text{M} + \text{K}]^+$. $^1\text{H-NMR}$ (CDCl_3) δ : 0.72 (3H, s, 18- CH_3), 0.97 (3H, s, 19- CH_3), 1.23 (3H, d, $J=6.2$ Hz, cym-6), 1.25 (3H, d, $J=6.1$ Hz, digt-6), 1.28 (3H, d, $J=6.3$ Hz, 21- CH_3), 1.29 (3H, d, $J=6.5$ Hz, hep-7), 1.31 (3H, d, $J=6.0$ Hz, can'-6), 1.33 (3H, d, $J=6.0$ Hz, can-6), 1.35 (3H, d, $J=6.5$ Hz, dig-6), 1.51 (3H, d, $J=6.8$ Hz, 6'- CH_3), 2.96 (1H, dd, $J=9.6$, 8.6 Hz, can'-4), 3.44, 3.46, 3.51, 3.63 (each 3H, s, OMe), 3.67 (1H, dd, $J=9.4$, 7.8 Hz, dig-2), 4.28 (1H, d, $J=7.8$ Hz, dig-1), 4.54 (1H, dd, $J=9.6$, 1.7 Hz, can-1), 4.57 (1H, dd, $J=9.6$, 1.7 Hz, can'-1), 4.73 (1H, d, $J=7.5$ Hz, hep-1a), 4.75 (1H, dd, $J=9.8$, 1.8 Hz, cym-1), 4.99 (1H, dd, $J=9.7$, 1.8 Hz, digt-1), 5.05 (1H, s, 1'- CH_3), 5.13 (1H, d, $J=7.5$ Hz, hep-1b), 5.35 (1H, m, 6-H), 5.78 (1H, d, $J=3.0$ Hz, 4'-H).

Periplocoside F (3)—Colorless powder, mp 195–198 °C, $[\alpha]_D^{20} +8.1^\circ$ ($c=0.07$, MeOH). $^1\text{H-NMR}$ (CDCl_3) δ : 0.72 (3H, s, 18- CH_3), 1.00 (3H, s, 19- CH_3), 1.20, 1.22, 1.25 (each 3H, d, $J=6.3$ Hz, cym-6), 1.29 (6H, d, $J=6.5$ Hz, 21- CH_3 and hep-7), 1.31 (3H, d, $J=6.0$ Hz, can-1), 1.35 (3H, d, $J=6.5$ Hz, dig-6), 3.43 ($\times 2$), 3.44 ($\times 2$), 3.52 (each 3H, s, OMe), 4.28 (1H, d, $J=7.7$ Hz, dig-1), 4.57 (1H, dd, $J=9.2$, 1.4 Hz, can-1), 4.74 (1H, d, $J=7.5$ Hz, hep-1a), 4.76, 4.77, 4.92 (each 1H, dd, $J=9.5$, 1.5 Hz, cym-1), 5.13 (1H, d, $J=7.5$ Hz, hep-1b), 5.35 (1H, m, 6-H).

Acetylation of Periplocosides E (11) and F (3)—The acetylations of 11 and 3 (5 mg) were carried out in the usual way with Ac_2O /pyridine, and afforded the same acetate as a colorless powder. $^1\text{H-NMR}$ (CDCl_3) δ : 0.72 (3H, s, 18- CH_3), 1.01 (3H, s, 19- CH_3), 1.17, 1.19, 1.21 (each 3H, dd, $J=6.5$ Hz, cym-6), 1.23 (3H, d, $J=6.5$ Hz, dig-6), 1.29 (6H, d, $J=6.5$ Hz, 21- CH_3 and hep-7), 1.31 (3H, d, $J=6.0$ Hz, can-6), 2.06, 2.10, 2.15 (each 3H, s, -OAc), 3.32, 3.42, 3.43, 3.44, 3.45 (each 3H, s, OMe), 4.41 (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-1a), 4.76 ($\times 2$), 4.92 (each 1H, dd, $J=9.6$, 1.5 Hz, cym-1), 5.10 (1H, dd, $J=10$, 8.0 Hz, dig-2), 5.13 (1H, d, $J=7.5$ Hz, hep-1b), 5.30 (1H, dd, $J=3.2$, 1.2 Hz, dig-4), 5.38 (1H, m, 6-H).

Acid Hydrolysis of Periplocosides J (1), K (2) and F (3)—Each sample (5 mg) was hydrolyzed with 0.05 N H_2SO_4 in 50% aqueous MeOH (2 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 ($\times 3$) and the CHCl_3 layer was washed with water. After removal of the organic solvent, the residue was purified by means of silica gel column chromatography to give Δ^5 -pregnene 3 β ,17 α ,20(S)-triol (5) or Δ^5 -pregnene 3 β ,17 α ,20(S)-triol 3-O-(4',6'-dideoxy-3'-O-methyl- Δ^3 -D-2'-hexosuloside) (10).¹⁾ The aqueous layer was neutralized with Amberlite IRA-94, and evaporated to dryness *in vacuo*. The residue showed the presence of cymarose (solv. 2, $R_f=0.62$; solv. 3, $R_f=0.45$), canarose (solv. 2, $R_f=0.37$; solv. 4, $R_f=0.26$); digitalose (solv. 2, $R_f=0.30$; solv. 3, $R_f=0.11$) and digitoxose (solv. 2, $R_f=0.35$; solv. 3, $R_f=0.34$) on silica gel TLC in comparison with authentic samples.

Periplocoside O (4)—Colorless powder, mp 103–106 °C, $[\alpha]_D^{20} -84.0^\circ$ ($c=0.05$, MeOH). $^1\text{H-NMR}$ (CDCl_3) δ :

0.73 (3H, s, 18-CH₃), 1.01 (3H, s, 19-CH₃), 1.30 (3H, d, $J=6.5$ Hz, 21-CH₃), 1.51 (6H, d, $J=6.0$ Hz, 6'-CH₃ and can-6), 3.49 (3H, s, OMe), 3.63 (3H, s, 3'-OMe), 3.74 (1H, q, $J=6.5$ Hz, 20-H), 4.58 (1H, dd, $J=10, 1.5$ Hz, can-1), 4.72 (2H, s, -OCH₂O-), 5.05 (1H, s, 1'-H), 5.78 (1H, d, $J=3.0$ Hz, 4'-H). The acid hydrolysis of **4** in the same manner as described above gave **5**.

Detection of Peroxides in Sugar Chains¹¹⁾—On TLC examination, each peroxide solution in chloroform revealed a characteristic purple spot when sprayed with a solution of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride (0.5 g) in a mixture of methanol (128 ml), water (25 ml) and glacial acetic acid (1 ml).

References and Notes

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- 9) While comparing the structures of periplocosides A and E^{2,3)} with those of periplosides C and A, as proposed by Oshima *et al.*,¹⁰⁾ isolated from the same plant, the combining form between heptulose and canarose of the corresponding sugar chain in previous papers^{2,3)} was shown to be of a different type, in spite of the same ¹H-NMR signal patterns, which is noteworthy.
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