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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

HIDEJI ITOKAWA,* JUNPING XU, and KOICHI TAKEYA

Tokyo College of Pharmacy, Horinouchi 1432–1, Hachioji, Tokyo 192–03, Japan

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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-*gluco*-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, $[\alpha]_D^{20}$ +24.13° (c=0.12, MeOH) gave the molecular formula $C_{61}H_{100}O_{23}$, based on its $[M+Na+H]^+$ peak at m/z 1224 and $[M+K+H]^+$ peak at m/z 1240 in the secondary ion mass spectrum (SIMS). The proton and carbon-13 nuclear magnetic resonance (^{1}H- 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-*gluco*-2-heptulose[2] [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 3β,17α,20(*S*)-triol (**5**)[1] as the aglycone and a disaccharide (**6**) which was confirmed to be methyl β-D-digitalopyranosyl-(1→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 ^{1}H–^{1}H 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-*gluco*-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-*gluco*-

2-heptulose.

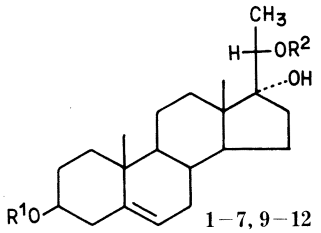
On the partial hydrolysis of **1** with 0.001 N H_2SO_4 in MeOH at room temperature, the hydrolyzate was found to contain **5**, periplocoside N [Δ^5-pregnene 3β,17α,20(*S*)-triol 20-β-D-canaropyranoside] (**7**)[3,6] and **6** by TLC comparison with authentic samples, as well as the product (**8**). Therefore, it was considered that canarose was the initial sugar in the sugar chain. The ^{1}H-NMR, ^{13}C-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 ^{1}H-, ^{13}C- and ^{1}H–^{1}H 2D-NMR spectra with those of **1** and D-

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

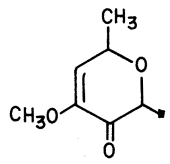
		1	**2**	**3**	**4**
1	t	37.25	37.37	37.29	37.38
2	t	31.87	29.41	31.89	29.41
3	d	71.74	78.62	71.76	78.64
4	t	42.30	38.56	42.34	38.52
5	s	140.71	140.35	140.75	140.37
6	d	121.63	122.01	121.63	121.96
7	t	31.94	31.92	31.98	31.94
8	d	31.67	31.92	31.71	31.94
9	d	49.64	49.72	49.69	49.74
10	s	36.66	36.74	36.77	36.74
11	t	20.58	20.58	20.61	20.59
12	t	36.90	36.89	36.93	38.44
13	s	45.34	45.37	45.37	45.38
14	d	51.08	51.12	51.11	51.14
15	t	23.48	23.47	23.50	23.49
16	t	30.97	31.00	31.00	31.02
17	s	85.45	85.47	85.46	85.48
18	q	14.14	14.14	14.15	14.15
19	q	19.40	19.35	19.40	19.36
20	d	85.45	85.47	85.46	82.86
21	q	17.99	18.01	18.00	18.07
1′	d		97.30		97.32
2′	s		185.87		185.91
3′	s		147.85		147.88
4′	d		118.49		118.49
5′	d		68.89		68.89
6′	q		23.01		23.03
OMe	q		54.98		54.97
Canarose					
1	d	100.82	100.82	100.82	100.80
2	t	38.40	38.42	38.41	37.76
3	d	77.03	77.02	77.03	81.45
4	d	79.19	79.22	79.22	75.35
5	d	69.95	69.96	70.00	71.79
6	q	17.06	17.06	17.06	17.01
					96.98 t (–OCH$_2$O–)
					55.74 q (OMe)

		1	**2**	**3**
Heptulose				
1	t	86.38	86.39	86.39
2	s	113.70	113.72	113.73
3	t	36.66	36.74	36.77
4	d	78.28	78.31	78.31
5	d	82.61	82.64	82.64
6	d	69.80	69.82	69.82
7	q	17.99	18.01	18.00
OMe	q	57.59	57.59	57.68
Digitoxose or cymarose				
1	d	98.50	98.50	98.50
2	t	38.40	38.45	36.77
3	d	69.21	68.89	77.69
4	d	82.04	82.05	82.52
5	d	66.78	66.80	68.88
6	q	17.83	17.83	18.24
OMe	q			58.05
Canarose or cymarose				
1	d	100.43	100.46	99.72
2	t	38.40	38.56	35.55
3	d	70.53	70.55	77.65
4	d	85.45	85.48	82.52
5	d	69.40	69.42	68.49
6	q	17.83	17.83	18.24
OMe				58.05
Cymarose				
1	d	99.29	99.32	99.78
2	t	35.45	35.49	35.25
3	d	77.34	77.35	77.34
4	d	82.61	82.64	83.05
5	d	68.25	68.28	68.45
6	q	18.23	18.22	18.24
OMe	q	58.51	58.52	58.20
Digitalose				
1	d	104.62	164.60	104.62
2	d	70.52	70.55	70.79
3	d	82.95	82.85	83.05
4	d	67.79	68.01	68.11
5	d	70.67	70.72	70.79
6	q	16.49	16.49	16.49
OMe	q	57.69	57.69	57.68

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.



1–7, 9–12



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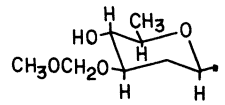

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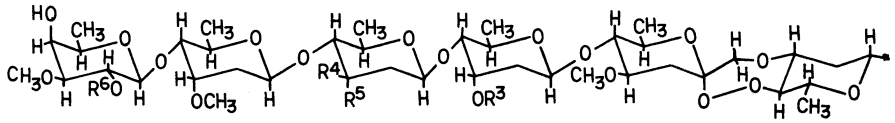

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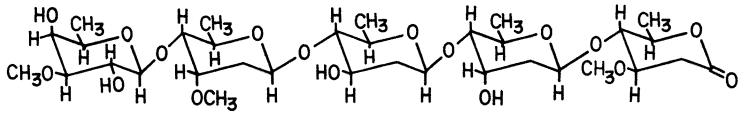

8

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→4)-β-D-cymaropyranoside
7: $R^1=H$, $R^2=$β-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: $R^1=I$, $R^2=$β-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→4)-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→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 3β,17α,20(*S*)-triol 20-*O*-β-D-digitalopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→5)-3,7-dideoxy-4-*O*-methyl-α-D-*gluco*-2-heptulopyranosyl-(2—4)-dioxy-(1→3)-β-D-canaropyranoside.

Periplocoside K (**2**), colorless powder, mp 208—212 °C, $[\alpha]_D^{20}$ −4.76° ($c=0.08$, 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 3β,17α,20(*S*)-triol 3-*O*-(4′,6′-dideoxy-3′-*O*-methyl-$\Delta^{3'}$-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 characteristics 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 ^{1}H- 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-$\Delta^{3'}$-D-2′-hexosulose moiety. Therefore, **2** was 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*-(β-D-digitalopyranosyl-(1→4)-β-D-cymaropyranosyl-(1→4)-β-D-canaropyranosyl-(1→4)-β-D-digitoxopyranosyl-(1→5)-3,7-dideoxy-4-*O*-methyl-α-D-*gluco*-2-heptulopyranosyl-(2—4)-dioxy-(1→3)-β-D-canaropyranoside).

Periplocoside F (**3**), colorless powder, mp 195—198 °C, $[\alpha]_D^{20}$ +8.1° (*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 ^{1}H- 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-3β,17α,20(*S*)-triol 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 O (**4**), colorless powder, mp 103—106 °C, $[\alpha]_D^{20}$ −84.0° (*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 ^{1}H- 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-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).

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 (^{1}H) 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 F_{254} (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° (c=0.12, MeOH). SIMS m/z: 1224 $[M(C_{61}H_{100}O_{23})+Na+H]^+$, 1240 $[M+K+H]^+$. ^{1}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-3β,17α,20(S)-triol (**5**) (solv. 3, Rf=0.51, solv. 5, Rf=0.50), periplocoside N (**7**) (solv. 3, Rf=0.34, solv. 5, Rf=0.23), and methyl β-D-digitalopyranosyl-(1→4)-β-D-cymaropyranoside (**6**) (solv. 3, Rf=0.47, solv. 5, Rf=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° (c=0.05, $CHCl_3$). ^{1}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}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° (c=0.08, MeOH). SIMS m/z: 1363 $[M(C_{68}H_{108}O_{26})+Na]^+$, 1379 $[M+K]^+$. ^{1}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° (c=0.07, MeOH). ^{1}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 (×2), 3.44 (×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}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 (×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 $CHCl_3$ (×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 3β,17α,20(S)-triol (**5**) or Δ^5-pregnene 3β,17α,20(S)-triol 3-*O*-(4′,6′-dideoxy-3′-*O*-methyl-$\Delta^{3'}$-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, Rf=0.62; solv. 3, Rf=0.45), canarose (solv. 2, Rf=0.37; solv. 4, Rf=0.26); digitalose (solv. 2, Rf=0.30; solv. 3, Rf=0.11) and digitoxose (solv. 2, Rf=0.35; solv. 3, Rf=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° (c=0.05, MeOH). ^{1}H-NMR ($CDCl_3$) δ:

0.73 (3H, s, 18-CH_3), 1.01 (3H, s, 19-CH_3), 1.30 (3H, d, J=6.5 Hz, 21-CH_3), 1.51 (6H, d, J=6.0 Hz, 6′-CH_3 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, $-OCH_2O-$), 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 Chains[11]——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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