PERIPLOSIDES A, B AND C, STEROIDAL GLYCOSIDES OF PERIPLOCA SEPIUM ROOT-BARKS[1]

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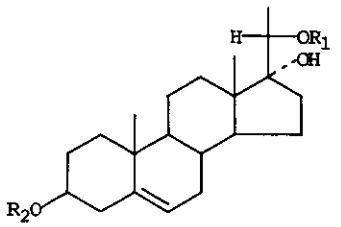
Abstract ——Three new steroidal glycosides, periplosides A, B and C, among which periploside A exhibited anticomplementary activity, have been isolated from Periploca sepium root-barks. The structures of periplosides A, B and C have been elucidated as shown in formulas **1**, **2** and **3**, respectively, on the basis of chemical and spectroscopic evidence.

The crude drug "Hoku-gokahi", prepared from the root barks of Periploca sepium Bunge (Asclepiadaceae), has been employed as a tonic in the Oriental system of medicines. Literature survey so far reported mainly the isolation and structure elucidation of steroidal glycosides and oligosaccharides from the root-barks of this plant.[2] During the course of the screening for active principles of the Oriental medicinal plants which have anticomplementary activity, we have found that the methanolic extract of Periploca sepium exhibited significant anticomplementary activity. Activity-guided fractionation of the methanolic extract afforded an active fraction which on further purification yielded three novel steroidal glycosides, now termed as periplosides A, B and C. In this paper, we wish to report the structure determination of these periplosides by means of chemical reactions and spectroscopic data.

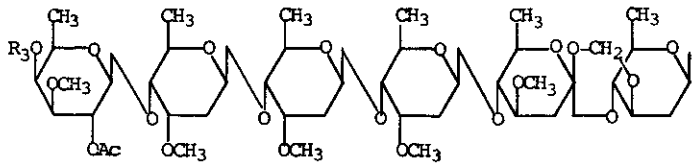
Periploside A, colorless prisms, mp 225-226°C, $[\alpha]_D$ +15.7° (c 0.27, MeOH), was shown to have the molecular formula $C_{65}H_{106}O_{24}$ from the results of its FD mass (*m/z 1294 (M^++Na+H) and 1271 (M^++H)*) and its ^{13}C nmr spectroscopy (Tables 1 and 2).[3] Since periploside A was suggested to be a steroidal glycoside bearing 2-deoxysugars by positive Liebermann-Burchard and Keller-Kiliani reactions, it was subjected to acid hydrolysis with 0.05N sulfuric acid in aqueous dioxane to liberate an aglycone, which was identified as Δ^5-pregnene-3β,17α,20α-triol (**4**),[4] along with D-canarose, D-cymarose and 4-O-(2-O-acetyl-β-D-digitalopyranosyl)-D-cymaropyranose.[5]

In order to clarify the location of the sugar moiety, periploside A was acetylated with acetic anhydride in pyridine to yield a diacetate (**5**) (*^{13}C nmr: δ 20.8 and 21.4 (each q) and 170.3 and 170.7 (each s); ^1H nmr: δ 2.00 and 2.11 (3H each s)*) which was, in turn, hydrolyzed with 0.5N sulfuric acid in aqueous dioxane to afford a monoacetylated derivative (**6**) as well as canarose, cymarose and 2,4-di-O-acetyldigitalose. Compound **6** differed from **4** in that the ^1H nmr signal at δ 4.55 (1H m) assigned to the C-3 hydrogen in the former was shifted downfield as compared to the corresponding one at δ 3.54 (1H m) in the latter, indicating **6** to be a 3-O-acetyl derivative of **4**. Thus, it became obvious that the sugar residue composed of canarose, cymarose and 2,4-di-O-acetyldigitalose was linked to C-17 and/or C-20 hydroxyl group of periploside A.

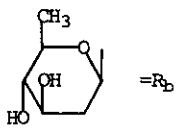
For the settlement of the structure of the sugar moiety and its attachment, periploside A was hydrolyzed with 0.05N sulfuric acid in methanol to give glycoside E (**7**)[6] as well as two other products (**8** and **9**). In the ^{13}C nmr spectrum of **7** (Py-d_5), it still showed signal at δ 85.4 (s) assigned to C-17, the resonance position of which was the same as that of periploside A (δ 85.4 (s)), leaving only C-20 as a sugar binding site in periploside A.



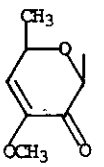
1: $R_1=R_a$, $R_2=R_3=H$
2: $R_1=R_b$, $R_2=R_c$
3: $R_1=R_a$, $R_2=R_c$, $R_3=H$
4: $R_1=R_2=H$
5: $R_1=R_a$, $R_2=R_3=Ac$
6: $R_1=H$, $R_2=Ac$
7: $R_1=R_b$, $R_2=H$
8: $R_1=R_d$, $R_2=H$



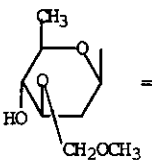
$=R_a$



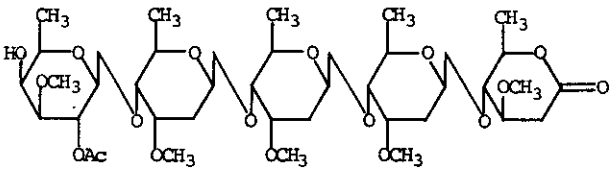
$=R_b$



$=R_c$



$=R_d$



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Compound **8** (FD-ms: m/z 509 (M^++H)) exhibited ^{1}H nmr signals due to the aglycone part (δ 0.66, 0.95 (3H each s, C_{18} and C_{19}-methyls), 1.23 (3H d, J 6.0 Hz, C_{21}-methyl), 3.47 (1H m, C_3-H) and 3.68 (1H q, J 6.0 Hz, C_{20}-H)) and those due to canarose moiety (δ 1.31 (3H d, J 6.0 Hz, C_6-H), 3.05 (1H t, J 9.0 Hz, C_4-H), 3.22 (1H dq, J 9.0 and 6.0 Hz, C_5-H), 3.33 (1H m, C_3-H) and 4.52 (1H dd, J 10.0 and 2.5 Hz, C_1-H)). In addition to these, the ^{1}H nmr spectrum of **8** showed a methoxyl signal at δ 3.38 (3H s) and a geminally coupled AB quartet signal centered at δ 4.62 (J 7.2 Hz). The latter signal pointed to the presence of a methylene group flanked between two oxygen atoms, which was further substantiated by the resonance position (δ 97.1 (t)) and the large $^1J_{CH}$ coupling constant (J 162 Hz)[7] of the ^{13}C nmr signal. Moreover, only the ^{1}H nmr signal due to the C-3 hydrogen of the canarose part of **8** was found shifted upfield as compared to that of **7**, suggesting that a methoxymethyl group is attached to the C-3 hydroxyl group of the canarose moiety of **8**. This was supported by the long-range ^{13}C-^{1}H couplings between the methylene carbon signal at δ 97.1 and the methoxyl hydrogen signal at δ 3.38, and between the same carbon signal and the signal at δ 3.33 due to the C-3 carbinyl hydrogen of the canarose part.

Compound **9** (FD-ms: m/z 833 (M^++K), 817 (M^++Na) and 794 (M^+)) showing thirty-seven carbon signals in its ^{13}C nmr spectrum was thought to be an oligosaccharide derived from the sugar moiety of

Table 1. Carbon-13 data of aglycone moieties of **1**, **2**, **3**, **5**, **7** and **8**.

	1	**2**	**3**	**5**	**7***	**8**
C-1	37.3	37.4	37.4	36.9	37.9	37.3
C-2	31.7	29.4	29.4	27.7	32.4	31.7
C-3	71.7	78.6	78.6	74.0	72.1	71.8
C-4	42.3	38.5	38.6	38.1	43.5	42.3
C-5	140.7	140.4	140.3	139.5	142.0	140.9
C-6	121.6	122.1	122.0	122.5	121.2	121.7
C-7	31.9	32.0	31.9	30.9	31.6	31.9
C-8	32.0	32.0	31.9	31.8	32.3	32.0
C-9	49.7	49.7	49.7	49.5	50.4	49.7
C-10	36.5	36.8	36.7	36.5	36.9	36.6
C-11	20.6	20.6	20.6	20.5	21.0	20.6
C-12	31.0	31.0	31.0	31.8	32.0	31.0
C-13	45.3	45.4	45.4	45.3	46.0	45.4
C-14	51.1	51.2	51.1	51.0	51.5	51.2
C-15	23.5	23.5	23.5	23.4	24.0	23.6
C-16	36.9	38.6	36.9	36.9	37.9	38.4
C-17	85.4	85.6	85.5	85.3	85.4	85.4
C-18	14.1	14.2	14.1	14.1	14.2	14.2
C-19	19.4	19.4	19.3	19.3	19.7	19.5
C-20	83.0	83.1	83.0	83.0	83.0	82.8
C-21	18.0	17.8	18.0	18.2	18.8	18.1
OAc				170.7		
				21.4		

* measured in Py-$\underline{d}_5$.

periploside A judged from the number of carbinyl carbon signals. The ^{1}H nmr spectrum of **9** disclosed one set of signals at δ 1.38 (3H d, $\underline{J}$ 6.0 Hz), 2.67 (1H dd, $\underline{J}$ 15.0 and 3.3 Hz), 2.72 (1H dd, $\underline{J}$ 15.0 and 2.7 Hz), 3.54 (1H dd, $\underline{J}$ 7.8 and 7.0 Hz), 3.94 (1H m) and 4.10 (1H m), which, along with the presence of a carbonyl carbon signal at δ 169.3 (s) and the fact that oligosaccharides C_1, D_1, F_1 and F_2 were isolated from the same plant,[1] indicated **9** to be an oleandronic acid-δ-lactone derivative. Further, a mass fragment ion peak at $\underline{m/z}$ 635 (M^+-δ-lactone) implied that this δ-lactone moiety was present at one terminal. The detailed decoupling experiments performed in the ^{1}H nmr spectrum of **9** revealed the presence of another set of signals at δ 2.00 (3H s), 3.25 (1H dd, $\underline{J}$ 10.0 and 3.0 Hz), 4.32 (1H d, $\underline{J}$ 8.0 Hz) and 5.02 (1H dd, $\underline{J}$ 10.0 and 8.0 Hz), the chemical shifts and the coupling patterns of which demonstrated that a 2-$\underline{O}$-acetyldigitalose was present in **9**. Besides the above functionalities, three moles of 2,6-dideoxysugars were easily identified by the presence of three pairs of secondary methyl, methoxyl and anomeric hydrogen signals in the ^{1}H nmr spectrum of **9**. These findings, together with the results of acid hydrolysis of periploside A and **5**, indicated that **9** was a saccharide which was composed of oleandronic acid-δ-lactone and 2-$\underline{O}$-acetyldigitalose as terminal units having three moles of inner cymaroses. The modes of glycoside linkages of cymarose units and 2-$\underline{O}$-acetyldigitalose unit were established as β from the coupling constants ($\underline{J}$ 10.0 and 2.5 Hz) of the anomeric hydrogen signals of three cymaroses and that ($\underline{J}$ 8.0 Hz) of 2-$\underline{O}$-acetyldigitalose in the ^{1}H nmr spectrum of **9**.

In the ^{13}C nmr spectrum of periploside A, a characteristic low field signal appeared at δ 113.7 (s), while no corresponding signal was observed in the ^{13}C nmr spectra of the other partially acid hydrolyzed products (**7**, **8** and **9**), indicating that an orthoester functionality was present in the C-20 side chain of periploside A. Further, the formation of the acetate (**5**) and the partially acid hydrolyzed products (**7**, **8** and **9**) was reasonably accounted for from the cleavage of this orthoester group, which was likely to be formed between the lactone carbonyl of **9** and the canarose moiety of **8**. On the basis of the above chemical and spectral evidence, periploside A was framed to have the structure **1**.

Table 2. Carbon-13 data of sugar moieties of **1**, **2**, **3**, **5**, **7**, **8** and **9**.

	1	**2**	**3**	**5**	**7***	**8**	**9**
can.							
C-1	100.8	100.9	100.8	100.7	102.5	100.8	
C-2	38.4	39.4	38.4	38.3	41.2	37.8	
C-3	77.1	71.8	77.1	77.0	73.0	81.6	
C-4	79.2	77.6	79.2	79.1	78.6	75.3	
C-5	70.0	71.7	70.0	69.9	71.3	71.7	
C-6	17.1	17.1	17.0	17.0	18.2	17.1	
OCH_2O	86.4		86.4	86.3		97.3	
OMe						55.8	
ole.							
C-1	113.7		113.7	113.6			169.3
C-2	36.7		36.8	36.9			31.9
C-3	78.3		78.3	78.3			75.9
C-4	82.7		82.7	82.4			79.6
C-5	69.8		69.8	69.7			75.1
C-6	18.3		18.2	18.0			18.0
OMe	57.7		57.7	57.7			55.8
cym.							
C-1	98.5		98.5	98.4			98.8
C-2	36.0		36.0	35.5			35.1
C-3	77.7		77.7	77.6			77.1
C-4	82.5		82.6	82.4			81.3
C-5	68.9		68.9	68.8			67.9
C-6	18.3		18.2	18.2			17.2
OMe	58.0		58.0	58.0			57.3
cym.							
C-1	99.7		99.7	99.6			98.7
C-2	35.5		35.5	35.5			34.7
C-3	76.6		77.1	76.2			75.6
C-4	82.5		82.5	82.4			81.5
C-5	68.4		68.4	68.3			67.5
C-6	18.2		18.2	18.2			17.0
OMe	58.0		58.0	57.8			57.0
cym.							
C-1	99.8		99.7	99.6			98.6
C-2	35.3		35.3	35.2			34.4
C-3	77.1		77.1	77.0			76.2
C-4	83.7		83.6	83.8			82.6
C-5	68.1		68.1	68.3			67.1
C-6	18.2		18.2	18.0			17.0
OMe	58.6		58.6	58.1			57.6
dig.							
C-1	102.5		102.6	102.4			101.5
C-2	70.9		70.9	70.8			69.9
C-3	81.6		81.6	80.0			80.6
C-4	68.0		68.1	69.2			67.0
C-5	70.4		70.4	68.0			69.4
C-6	16.5		16.5	16.5			15.5
OMe	57.4		57.4	57.6			56.4
OAc	169.4		169.5	169.3			168.4
	21.0		21.0	20.9			20.0
OAc				170.3			
				20.8			
hex.							
C-1		97.3	97.2				
C-2		186.0	185.9				
C-3		147.9	147.8				
C-4		118.6	118.5				
C-5		68.9	68.9				
C-6		23.0	23.0				
OMe		55.0	55.0				

* measured in Py-$\underline{d}_5$.

Periploside B, colorless prisms, mp 146-147°C, $[\alpha]_D$ -71.6° (c 0.25, MeOH), was found to have the molecular formula $C_{34}H_{52}O_9$ from its FD-ms (m/z 604 (M^+)) and its ^{13}C nmr spectrum (Tables 1 and 2). Periploside B gave positive Liebermann-Burchard and Keller-Kiliani reactions for steroidal glycoside having 2-deoxysugar. The ^1H nmr spectrum of periploside B exhibited signals for two tertiary methyls (δ 0.65 and 0.93 (3H each s)), one secondary methyl (δ 1.24 (3H d, J 6.0 Hz)), two carbinyl hydrogens (δ 3.60 (1H m) and 3.68 (1H q, J 6.0 Hz)) and one olefinic hydrogen (δ 5.28 (1H brs)) which were virtually identical to those of **7** except the C-3 carbinyl hydrogen signal. In addition to these signals, the signals assigned to canarose moiety appeared at δ 1.27 (3H d, J 6.0 Hz), 3.04 (1H t, J 9.0 Hz), 3.22 (1H dq, J 9.0 and 6.0 Hz), 3.50 (1H m) and 4.55 (1H dd, J 9.0 and 2.0 Hz). Comparison of the ^{13}C nmr data of periploside B with those of **7** revealed that they had identical signals except for C-2, C-3 and C-4 of the aglycone moiety. From these data, it was concluded that periploside B is a congener of glycoside E (**7**) bearing a C-3 side chain.

The ^{13}C nmr spectrum of periploside B showed the presence of a carbonyl and a trisubstituted double bond in the C-3 side chain of the composition $C_7H_9O_3$ (ms: m/z 141). From this finding, together with the uv absorption maximum at 262 nm (log ε 3.48 in EtOH) these two groups were considered to be in conjugation. In the ^1H nmr spectrum of periploside B, signals due to an anomeric hydrogen and a carbinyl hydrogen appeared at δ 4.98 (1H s) and 4.64 (1H dq, J 3.0 and 6.0 Hz), respectively, the latter of which were found coupled with the olefinic hydrogen signal at δ 5.72 (1H d, J 3.0 Hz) and the secondary methyl signal at δ 1.45 (3H d, J 6.0 Hz). These spectral data suggested that the C-3 side chain consisted of a 4,6-dideoxy-3-O-methyl-Δ^3-2-hexosulose moiety. Further, the close resemblance of the ^1H and ^{13}C nmr signals of the hexosulosyl moiety of periploside B to those of affinoside O[8] demonstrated that they have identical stereochemistry at two chiral centers of this moiety. Accordingly, periploside B was shown to have the structure **2**.

Periploside C, colorless prisms, mp 194-195°C, $[\alpha]_D$ +1.3° (c 0.45, MeOH), responding to Liebermann-Burchard and Keller-Kiliani reactions like periplosides A and B, showed seventy-two carbon signals in its ^{13}C nmr spectrum (Tables 1 and 2). A comparative study of the ^1H and ^{13}C nmr spectra of periploside C with those of periplosides A and B clarified that periploside C has the same aglycone and C-3 side chain as periploside B, and furthermore, it has identical C-20 side chain like periploside A. Thus, periploside C was determined to have the structure **3**.

It should be noted that periplosides A and C bears orthoester groups in their molecules which are rather uncommon functional groups in natural products.

Among these periplosides, periploside A showed significant anticomplementary activity at the concentration of 1.0 mg/ml by the method of Mayer.[9]

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