Porwenins A and B, New Clerodane Diterpenoids from *Portulaca okinawensis*

Ayumi Ohsaki,*,† Mari Ogawa,† Yoji Imai,† Takakazu Shinzato,‡ Hideyuki Shigemori,§ and Jun'ichi Kobayashi*,§

Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, 101-0062, Japan, Faculty of Agriculture, University of the Ryukyus, Okinawa 905-1427, Japan, and Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

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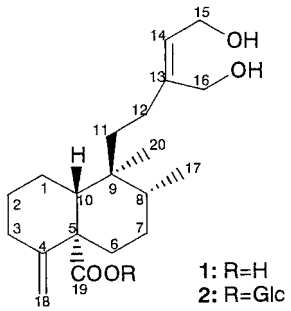
Two new clerodane-type diterpenes, porwenins A (**1**) and B (**2**), were isolated from *Portulaca okinawensis*, and the structures were elucidated by spectroscopic data.

From biosynthetic and chemosystematic points of view, we have studied the structures of diterpenoid constituents of *Portulaca* species.[1] Among them, *Portulaca okinawensis* (Portulacaceae) is a rare, peculiar plant, distributed on rock cliffs on the seashore of Okinawa Island. Recent investigation of extracts of *P. okinawensis* resulted in the isolation of two new clerodane diterpenes, porwenins A (**1**) and B (**2**). Here we describe the isolation and structure elucidation of **1** and **2**.



The molecular formula, $C_{20}H_{32}O_4$, of porwenin A (**1**) was established by HRFABMS. The IR spectrum suggested the presence of hydroxy (3348 cm^{-1}) and carboxylic (1710 cm^{-1}) groups. The gross structure of **1** was deduced from detailed analysis of the ^1H and ^{13}C NMR data (Table 1) aided by 2D NMR experiments ($^1H-^1H$ COSY, $^{13}C-^1H$ COSY, and HMBC). The ^1H and ^{13}C NMR data indicated that compound **1** possessed one carboxyl group, one trisubstituted olefin, one exomethylene, two hydroxy methylenes, two methyl groups, two sp^3 quaternary carbons, two methines, and seven methylenes. Since three of five unsaturations were accounted for, it was indicated that **1** contained two rings. The $^1H-^1H$ COSY spectrum revealed connectivities of C-1 to C-3 and C-10, C-6 to C-8 and C-17, and C-11 to C-12 (Figure 1). HMBC correlations (Figure 1) of H-3 to C-4, H-18 to C-3, C-4, C-5, and C-19 (δc 178.1), and H-10 to C-1 and C-19 suggested the presence of a cyclohexane ring (ring A), in which an exomethylene unit and a carboxyl group were attached to C-4 and C-5, respectively. The presence of another cyclohexane ring (ring B) with Me-17 and Me-20 at C-8 and C-9, respectively, was elucidated by HMBC correlations of H-6 to C-10 and C-8, H_3-17 to C-7, C-8, and C-9, and H_3-20 to C-9 and C-10. The side chain, a 1,4-dihydroxy-2-buten-2-ylethyl group, was attached to C-9 due to HMBC cross-peaks of H_3-20 to C-9 and C-11.

Table 1. ^1H and ^{13}C NMR Data for Porwenins A (**1**) and B (**2**) in CD_3OD[a]

	^1H		^{13}C	
position	**1**	**2**	**1**	**2**
1	1.63m, 2.30m	1.63m, 2.32m	22.6	22.5
2	1.93m	1.48m, 1.78m	29.0	29.6
3	2.17brd(13.4), 2.56m	2.15brd(13.4), 2.49dt(13.4,4.9)	34.1	34.0
4			153.6	152.6
5			52.3	52.9
6	1.30m, 2.51m	1.38m, 2.58dt(12.8,3.0)	36.1	35.6
7	1.50m	1.33m, 1.93m	29.9	29.1
8	1.58m	1.58m	38.2	38.2
9			40.9	41.0
10	1.33dd (12.8, 3.1)	1.36dd(12.8, 3.0)	52.6	53.2
11	1.46m, 1.56m	1.42m, 1.54m	37.6	37.6
12	1.88m, 2.24m	1.85m, 2.04m	28.3	28.4
13			143.6	143.6
14	5.45t (6.7)	5.45t (6.7)	127.3	127.3
15	4.13d (6.7)	4.13d(6.7)	58.8	58.8
16	4.09s	4.08s	60.3	60.2
17	0.82d (6.1)	0.82d(6.7)	16.3	16.3
18	4.76brs, 4.78brs[b]	4.85brs, 4.86brs	109.7	110.3
19			178.1	174.1
20	0.75s	0.74s	18.0	18.6
1′		5.45d(7.4)		95.6
2′		3.33m		74.2
3′		3.32m		78.8
4′		3.35m		71.0
5′		3.39m		78.6
6′		3.67dd(12.2,1.2), 3.80dd(12.2,4.3)		62.3

[a] 500 MHz for ^1H and 125 MHz for ^{13}C; *J* values (Hz) in parentheses. [b] The spectrum was measured in CD_3COCD_3.

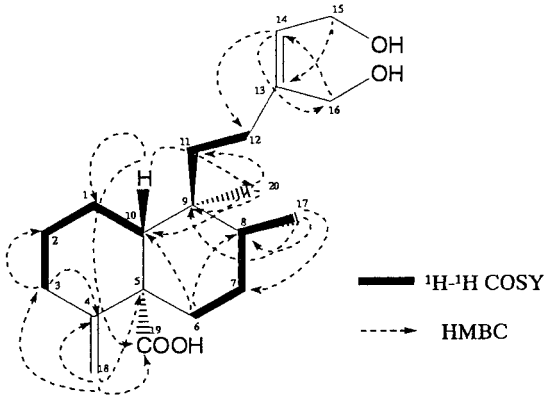


Figure 1. Selected 2D NMR correlations of porwenin A (**1**).

* Corresponding authors. Tel: +81-3-5280-8153. Fax: +81-3-5280-8005. E-mail: a-ohsaki.fm@tmd.ac.jp (A.O.). Tel: +81-11-706-4985. Fax: +81-11-706-4989. E-mail: jkobay@pharm.hokudai.ac.jp (J.K.).
† Tokyo Medical and Dental University.
‡ University of the Ryukyus.
§ Hokkaido University.

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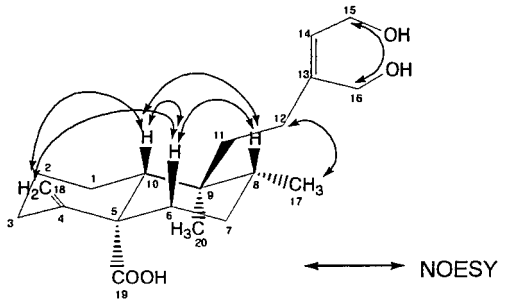

Figure 2. Selected NOESY correlations of porwenin A (**1**).

NOESY correlations (Figure 2) of H_2-18 to H-10, H-6b, H-10 to H-6b, H-8, and H-6b to H-8 indicated chair conformations of rings A and B, a *trans* relationship between rings A and B, α-orientations of Me-17, Me-20, and a carboxyl group at C-5, and *β*-orientation of H-10. Therefore, the structure and relative stereochemistry of **1** were assigned as shown.

The molecular formula, $C_{26}H_{42}O_9$, of porwenin B (**2**) was established by HRFABMS. The IR spectrum indicated the presence of hydroxy (3356 cm^{-1}) and ester (1734 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra of **2** were similar to those of porwenin A (**1**). Analysis of the ^1H and ^{13}C NMR data (Table 1) and the $^{13}C-^1H$ COSY spectrum of **2** provided five methines (all of which were bearing an oxygen atom) and one hydroxymethylene in addition to all the carbons of **1**. $^1H-^1H$ connectivities of C-1′ to C-6′ and the ^1H and ^{13}C chemical shifts (Table 1) of **2** suggested the presence of a glucose moiety. Furthermore, the anomeric proton signal at δ_H 5.45 (d, $J = 7.39$, H-1′) and the carbon signal at δ_C 95.6 (C-1′) having the $^1J_{CH}$ value of 163 Hz, which was deduced from INEPT-nondecoupling measurement,[2] revealed the *β*-configuration of the glycoside bond at C-1′. Comparison of the ^{13}C NMR data of **1** and **2** and the HMBC cross-peak of H-1′ to C-19 in **2** indicated that the 19-carboxyl group of **1** was glycosylated in **2**. Thus, the structure of porwenin B was assigned as **2**. The relative stereochemistry of **2** was elucidated to be the same as that of **1** by NOESY correlations (Figure 2).

Porwenins A (**1**) and B (**2**) possess in common a 1,4-dihydroxy-2-buten-2-ylethyl group at C-9, which is characteristic of the diterpenoids found in *Portulaca* plants, while the ring skeleton of both **1** and **2** is that of normal-type clerodane diterpenes without skeletal rearrangement such as the major constituents of *Portulaca* cv. Jewel.[3]

Experimental Section

General Experimental Procedures. IR spectra were obtained with a JASCO infrared microscope and Jansenn MFT-2000 spectrometer. Optical rotations were obtained with a JASCO DIP-370 polarimeter. HRFABMS measurements were carried out using a JEOL HX-110 mass spectrometer. ^1H and ^{13}C spectra, including 2D experiments, were measured using a JEOL α-500 NMR spectrometer. TMS and the resonance of residual CD_3OD (49.0 ppm) were used as internal reference for ^1H and ^{13}C NMR, respectively.

Plant Material. *P. okinawensis* was collected on the rock cliff of the seashore at Yomitan Village, Okinawa Island, in September 1999 and cultivated in Yokohama. This plant was identified by Dr. T. Shinzato (Faculty of Agriculture, University of the Ryukyus). A voucher specimen has been deposited at the Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University.

Extraction and Separation. The fresh whole plant (133.2 g) of *P. okinawensis* was extracted with MeOH, and the extract (4.2 g) was partitioned between hexane and 90% aqueous MeOH. The MeOH-soluble portion was partitioned with EtOAc and H_2O. The EtOAc-soluble materials were subjected to an ODS column (YMC-GEL, ODS-AM 120-S50, YMC co. LTD, Japan) with CH_3CN-H_2O (0:100 → 100:0) to give six fractions. The fraction eluted with CH_3CN-H_2O (40:60) was separated with CH_3CN-H_2O (20:80 → 80:20) by C_{18} HPLC (Capcell pak, UG-120, 3.0 × 250 mm, Shiseido; flow rate, 8 mL/min; detection, 210 nm) to afford porwenins A (**1**, 4.7 mg) and B (**2**, 7.5 mg).

Porwenin A (1): colorless amorphous solid; $[\alpha]^{23}_D$ +45.5° (*c* 0.24, MeOH); IR (neat) ν_{max} 3348, 1710 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRFABMS (negative) *m/z* 335.2247 $(M - H)^-$ (calcd for $C_{20}H_{31}O_4$, 335.2222).

Porwenin B (2): colorless amorphous solid; $[\alpha]^{23}_D$ +36.6° (*c* 0.37, MeOH); IR (neat) ν_{max} 3356, 1734 cm^{-1}; ^1H and ^{13}C NMR (Table 1); HRFABMS (positive) *m/z* 521.2712 $(M + Na)^+$ (calcd for $C_{26}H_{42}O_9Na$, 521.2727).

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References and Notes

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