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A DITERPENOID FROM *PORTULACA PILOSA*

AYUMI OHSAKI,* YOSHIHISA KASETANI,† YUKIHIRO ASAKA,† KOZO SHIBATA,‡ TAKASHI TOKOROYAMA‡ and TAKASHI KUBOTA†

Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, 2-3-10, Surugadai, Kanda, Chiyoda-ku, 101, Japan; †School of Medicine, Kinki University, Ohnohigashi, Osaka-sayama, Osaka, 589, Japan; ‡Faculty of Science, Osaka City University, Sumiyoshi-ku, Osaka, 558, Japan

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Key Word Index—*Portulaca pilosa*; Portulacaceae; diterpene; bicyclo[5.4.0]undecane skeleton; pilosanone C; biosynthesis; evolution.

Abstract—A new diterpenoid, pilosanone C, having a bicyclo[5.4.0]undecane skeleton, has been isolated from the aerial parts of *Portulaca pilosa*. Its structure was elucidated on the basis of spectral studies. Its biosynthesis and implication in chemosystematic evolution is discussed.

INTRODUCTION

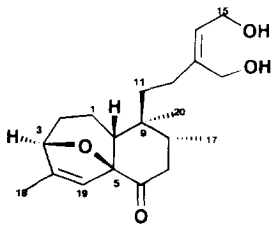
In view of our interest in biosynthesis and chemosystematic evolution, we have been studying the structures of the diterpenoid constituents of *Portulaca* plants. Previously, we reported on the structure elucidation of pilosanone A (**2**) and B (**3**) isolated as the major constituents from the aerial parts of *Portulaca pilosa* L. [1, 2]. These diterpenoids have the novel bicyclo[5.4.0]undecane skeleton [1–4] which represents a possible step on the pathway from the *ent*-labdadienyl cation to portulal (**5**) [5, 6]. Herein, we report on the isolation and structure elucidation of a new diterpenoid, a congener of **2** and **3**, occurring as a minor constituent in the aerial parts of *P. pilosa*.

RESULTS AND DISCUSSION

The chromatographic fractions of the ethyl acetate extract of *P. pilosa*, other than those containing pilosanone A (**2**) and B (**3**), were further separated by silica gel CC to give a new diterpenoid designated as pilosanone C (**1**).

Pilosanone C (**1**), mp 154.5–155.0 (EtOAc), $[\alpha]_D^{21}$ + 40.3 (MeOH; *c* 0.72), was assigned the molecular formula $C_{20}H_{30}O_4$ on the basis of the $[M]^+$ peak in the high-resolution-mass spectrum (*m/z* 334.2164, calcd: 334.2144). The IR spectrum showed the presence of hydroxyl and carbonyl groups at 3380 and 1710 cm^{-1}, respectively. Inspection of the ^1H and ^{13}C NMR spectra of **1** (Tables 1 and 2) indicated the presence of the 5-hydroxy-3-hydroxymethyl-3-pentenyl side-chain, a characteristic of the diterpenoids found in *Portulaca* plants, in addition to a tertiary methyl group (δ_H 0.87, *s*, H-20; δ_C 17.0, C-20) and a secondary methyl group (δ_H 0.95, *d*, J = 7.3 Hz, H-17; δ_C 15.8, C-17). The spectra revealed also the presence of an additional trisubstituted double bond system $-C(CH_3)=CH-$ [(δ_H 1.83, *d*, J = 1.3 Hz, H-18 and 5.81 *q*, J = 1.3 Hz, H-19; δ_C 143.5, *s*, C-4, 12.7 *q*, C-18 and 123.8, *d*, C-19)]. Thus, compound **1** had to be tricyclic with two carbocyclic rings and, on taking the molecular formula into consideration, an ether ring. Since one of the carbocyclic rings was presumed to be a cyclohexanone ring (IR), **1** was most likely to have the bicyclo[5.4.0]undecane skeleton common to the congeners, **2** and **3**. In the ^1H and ^{13}C NMR spectra the signal characteristics of the H-7 protons and the chemical shifts of the C-6 to C-10 signals were similar to those of the B-ring in **2**. However, the signal patterns ascribed to the protons and carbons of the A-ring in **1** differed considerably from those in **2**. The major differences were the presence of the signals at δ_C 83.8 (*d*) and 94.1 (*s*), instead of the methylene carbon of C-19 and methine carbon of C-5 found in **2**. These observation suggested an ether bridge at C-3 → C-5 in the A-ring. An additional feature in the ^{13}C NMR spectrum of **1** was that the resonances of C-1 and C-18 (vinylmethyl) were more shielded by 8.1 and 12.9 ppm, respectively, compared to those of **2**; a fact attributable to the anisotropy effect of the ether oxygen atom. In the ^1H NMR spectrum of **1**, the signal of the proton attached to the ether oxygen (H-3) was observed at δ4.47 as a broad singlet. The results of the ^1H and ^{13}C NMR analysis based on homonuclear spin decoupling (HNSD), DEPT, ^1H–^1H COSY, ^1H–^{13}C COSY and, HMBC [7] techniques established that the A-ring of **1** was a seven-membered ring similar to **2** and **3**, that the ether ring was located at C-3 → C-5, and that the ring olefin was present at C-4/C-19. Thus the

*Author to whom correspondence should be addressed.



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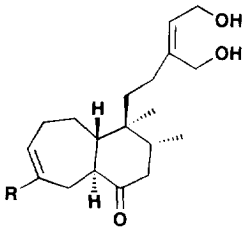
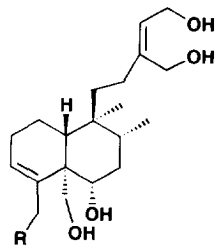


2 R=Me

3 R=CH_2OH



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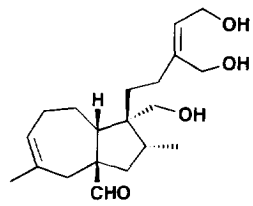
R=H or OH



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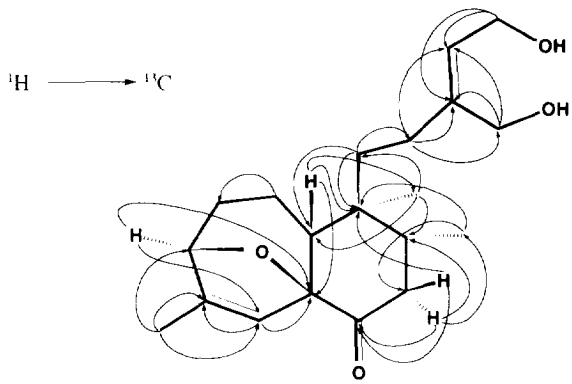

Fig. 1. HMBC correlations for compound **1**.

Table 1. ^1H NMR spectral data of compound **1** (500 MHz, CD_3OD, TMS)

H	δ (J, Hz)
1	1.65 *m*
2	1.50 *m*, 1.73 *m*
3	4.47 br s
7α	2.66 *t* (14.0)
7β	2.23 *dd* (14.0, 3.0)
8	2.00 *m*
10	1.88 *t* (7.4)
11	1.49 *m*
12	2.00 *m*
14	5.47 *t* (6.7)
15	4.12 *d* (6.7)
16	4.10 *s*
17	0.95 *d* (7.3)
18	1.83 *d* (1.3)
19	5.81 *q* (1.3)
20	0.87 *s*

A-ring constituted a rigid and strained shape. A W-type coupling was observed between the signals due to H-3 and H-1α (δ 1.65). In addition, there was a correlation between H-19 and H-20 in the NOESY spectrum. These lines of evidence suggested the presence of the ether ring on the same face of the molecule as H-10 and the side-chain. Figure 1 shows the HMBC correlations

Table 2. ^{13}C NMR spectral data of compounds **1–3**

	1 (CD_3OD, 125 MHz)	**2** ($CDCl_3$, 100 MHz)	**3** (CD_3OD, 100 MHz)
1	18.7	26.8	28.0
2	24.2	26.6	28.2
3	83.8	126.1	128.8
4	143.5	137.5	149.1
5	94.1	49.1	51.3
6	208.5	211.2	213.9
7	45.3	46.4	47.9
8	40.0	38.5	40.4
9	39.7	40.1	41.7
10	48.3	51.7	53.7
11	36.1	35.3	37.0
12	28.4	28.2	29.0
13	143.1	143.6	143.5
14	127.6	126.1	127.8
15	58.7	58.6	59.4
16	60.2	61.0	60.9
17	15.8	15.7	17.4
18	12.7	25.6	68.7
19	123.8	31.8	29.1
20	17.0	16.4	16.7

for **1**. From all of the above spectral data pilosanone C was concluded to have the bicyclo[5.4.0]undecane structure **1**.

Pilosanone C showed negative CD bands at 230 ($\Delta\varepsilon$ – 3.45, EtOH) and 308 nm ($\Delta\varepsilon$ – 0.12, EtOH). The latter band based on n $\rightarrow \pi^*$ has a weak intensity. This result supports the proposal that pilosanone C has the stereostructure shown, but it is not good evidence of the absolute configuration [8]. However, pilosanone C presumably has the absolute structure shown in **1** by analogy with its congener pilosanone A (**2**).

In a previous report [2], we proposed a linear pathway rather than a ramified one for the biosynthesis of the diterpenoid constituents in *Portulaca* plants based on the co-occurrence of bicyclo[5.4.0]undecane diterpenes and 19-oxygenated *trans*-clerodane compounds in *P. pilosa*, which were found in the aerial parts and the roots, respectively. The first stage is the formation of a cyclo-

propylmethylene cation through extrusion of the C-19 hydroxy group in a pilosanol-type precursor (**4**) by the participation of a C-3/C-4 π-bond and its subsequent collapse to give a bicyclo[5.4.0]undecane diterpene. In the second stage, this compound is converted to a bicyclo[5.3.0]undecane diterpene, represented by portulal (**5**), through a Favorskii-type rearrangement. The oxygen function at C-5 in **1** might be significant, since such a substituent would act as a suitable leaving group in the rearrangement reaction. Thus the occurrence of **1** in *P. pilosa* lends further support to our postulation on the biosynthesis of portulal (**5**) and the evolutionary transition to it in the *Portulaca* species.

EXPERIMENTAL

^{1}H NMR: 500 MHz with TMS an int. standard; ^{13}C NMR: 125 MHz; EIMS: ionization voltage 70 or 22 eV.

Plant material. Portulaca pilosa L. was collected at Iriomote Island, Japan, and cultivated in the plant garden at the Faculty of Science, Osaka City University.

Extraction and isolation of diterpenoid. The aerial parts of the fresh plants (3.4 kg) were ground with MeOH and kept at room temp. for several weeks. After filtration, the filtrate was evapd. This extract was partitioned successively with hexane (63 g) and EtOAc (47 g). The EtOAc extract was chromatographed on silica gel. Elution with $CHCl_3$–MeOH (19:1) gave frs containing pilosanone A–C and these were sepd by repeated chromatography (Merck Lobar column RP-18, MeOH–H_2O, 3:2; silica gel 230–400 mesh; $CHCl_3$–MeOH, 99:1; prep. TLC, CH_2Cl_2–Et_2O–MeOH, 17:17:6 affording pilosanone C (**1**), which was recrystallized from EtOAc (32.6 mg).

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