Portulene Acetal, a Novel Minor Constituent of *Portulaca grandiflora* with Significance for the Biosynthesis of Portulal

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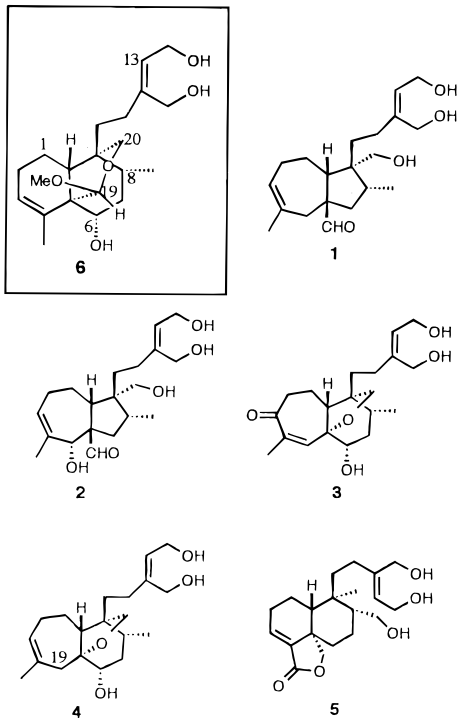
A new clerodane diterpenoid (**6**) with a caged hemiacetal ring has been isolated as a minor constituent from *Portulaca grandiflora* and its structure elucidated by extensive spectral studies. The compound might be of significance in the biosynthesis and in the chemosystematics of constituents of the genus *Portulaca*. The structural features of **6** provide important clues for the proposal of a chemically reasonable scheme for the biosynthesis of portulal (**1**).

Portulal (**1**) is a diterpene with a unqiue bicyclo[7.5]-decane ring system, isolated from *Portulaca grandiflora* Hook. (Portulacaceae) as a plant-growth regulator.[1] As a result of our interest in both its biosynthesis[2] and the chemosystematics of *Portulaca* species, we have carried out an extensive investigation on the constitutents of *P. grandiflora* and related species.[3] We have characterized already six compounds[4] with a perhydroazulene skeleton and three compounds (e.g., **3**, **4**)[5] with a novel bicyclo[7.6]undecane ring system from *P. grandiflora*.



The isolation of portulide (**5**),[6] a clerodane diterpene, was reported by us, but subsequent cultivation studies disclosed that this compound (renamed as portulide A) was originally a constituent of *Portulaca* cv. Jewel, and contamination of the commercial seeds of *P. grandiflora* with those of the latter variety caused an incorrect conclusion. In a continuation of studies on the minor constituents of *P. grandiflora*, we have confirmed the occurrence of a new clerodane diterpene, **6**, designated as portulene acetal, which might have significant implications in the biosynthesis of portulal (**1**).

Table 1. NMR Data for Portulene Acetal (**6**) in CD_3OD

position	^1H	*J* (Hz)	^{13}C	HMBC with H no.
1a	1.53		20.28	
1b	1.94			
2	2.00		27.25	
3	5.50	br d (5.5)	126.50	1,18
4			138.77	18
5			47.03	1,3,7,18
6	3.53	dd (6.0,11.0)	77.20	7,19
7a	1.88		42.37	8,17
7b	2.02			
8	1.74	br quin. (6.7)	36.14	7,17,20
9			37.63	7,8,11,12,17
10	1.17		44.46	11,19,20
11a	1.17		30.21	12
11b	1.25	dt (5.0,14.0)		
12a	1.88		27.19	11,14,16
12b	2.01			
13			143.23	15,16
14	5.48	br t (6.7)	127.39	12,15,16
15	4.15	d (6.7)	58.71	
16	4.11	d (3.1)	60.14	14
17	0.96	d (6.7)	15.75	8,7
18	1.82	br s	22.71	3
19	4.88	s	100.36	6,20,21
20a	3.34	br d (11.6)	62.89	8,19
20b	3.48	br d (11.6)		
21	3.28	s	55.75	19

Portulene acetal (**6**) was obtained as colorless oil, $[\alpha]^{25}_D$ +74.9° (*c* 0.25, MeOH), after repeated chromatography on Si gel columns and ODS columns of fractions obtained by successive partition with Et_2O and EtOAc of the MeOH extract of fresh whole plants of *P. grandiflora*. Compound **6** was assigned the molecular formula $C_{21}H_{34}O_5$ on the basis of the $[M]^+$ peak in the HREIMS. In the IR spectrum, **6** exhibited hydroxyl absorption at 3400 cm^{-1}. The signals in the ^1H- and

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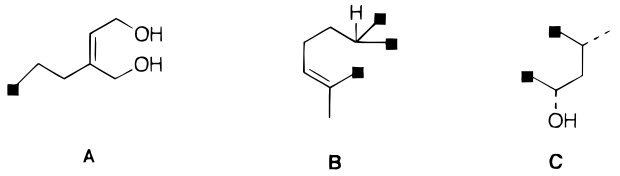


Figure 1. Structural segments of compound **6** deduced on the basis of NMR experiments.

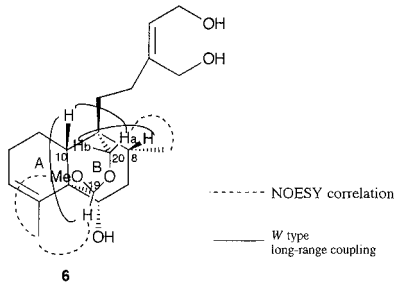


Figure 2. Significant NOESY correlations and *W*-type long-range couplings of **6** in the ^{1}H NMR spectrum.

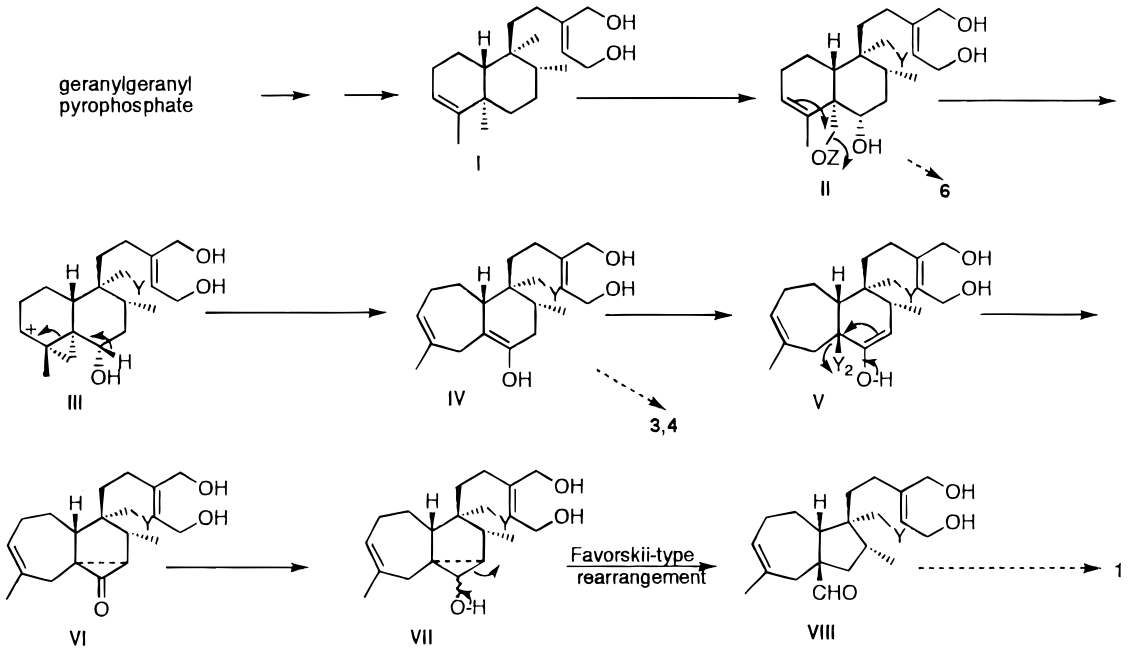
^{13}C-NMR spectra (δ 3.28 and 55.75, respectively; see Table 1) indicated that an extra carbon atom on a diterpene skeleton could be attributed to a methoxy group. With the aid of ^{1}H−^{1}H COSY, DEPT, and ^{1}H−^{13}C COSY spectra, the presence of segments A−C was deduced (Figure 1). In addition, the presence of an oxymethylene group was revealed from the appearance of an AB quartet centered at δ 3.34 and 3.48 in the ^{1}H-NMR spectrum and a signal at δ 62.89 (t) in the ^{13}C-NMR spectrum. Both of the H_a-20 and H_b-20 signals were shown to have *W*-coupling with an angular proton, H-10, and a methine proton, H-8, respectively, by long-range ^{1}H−^{1}H COSY, HOHAHA, and homonuclear spin decoupling measurements being conducted. These structural features resembled closely those of portulene (**4**). The difference concerned ring A, which in **6** lacked an additional methylene group (C-19) adjacent to the trisubstituted double bond present in the case of **4**, as seen in segment B. Instead, the presence of an acetal methine group in **6** was indicated from the signals at δ 4.88 and 100.36 in the ^{1}H-NMR and ^{13}C-NMR spectra, respectively. These facts suggested that the C-19 carbon atom was part of an acetal ring structure with C-20, involving the methoxy group. HMBC correlations for **6**, which support all of the aforementioned deductions on the structure shown as **6** are given in Table 1. Thus, portulene acetal (**6**) was concluded to have a clerodane skeleton. The stereochemistry of the methoxy group was defined by 2D NMR measurements: *W*-coupling was observed between the acetal proton (H-19) and the angular proton (H-10) in the long-range ^{1}H−^{1}H COSY spectrum, and the H-19 and methoxy protons showed correlations with H-18 (vinyl methyl protons) in the NOESY spectrum, respectively, which indicated the relative configuration of **6** (Figure 2). Portulene acetal (**6**) might possibly be an artifact derived from the reaction of the corresponding hemiacetal, with the MeOH used in the extraction.

Thus, the co-occurrence in *P. grandiflora* of the clerodane diterpene **6** with a [6.6]-ring in addition to compounds with [7.6]- and [7.5]-ring systems has been confirmed. This finding supports the biosynthetic pathway, proposed by us, involving clerodane diterpenes in a linear path rather than in divergent one.[3d] Furthermore, the 6,19-oxygenation observed in **6** might be important in the mechanism of ring alteration in the biosynthetic process. With this consideration the detailed biosynthetic pathway shown in Scheme 1 is proposed. The intermediate II, a possible precursor of **6**, is transformed to a cyclopropylium cation III by homoallylic participation of C3−C4 π-bond, which collapses to the [7.6]-bicyclo-intermediate IV. The ensuing Favorskii-type rearrangement would lead to the formation of portulal with the [7.5]-bicyclic ring system.

Experimental Section

General Experimental Procedures. The following instruments were used: JASCO FT/IR-7000 (IR), JASCO DIP-370 polarimeter (optical rotation), JEOL JMS-

Scheme 1. Proposed Biosynthetic Pathway of Portulal (**1**)



HX-100 mass spectrometer (HRMS), and JEOL α-500 NMR spectrometer (^{1}H and ^{13}C NMR).

Plant Material. The material studied was cultivated from commerical seeds of *Portulaca grandiflora* at the botanical garden of the parks section of Osaka City. Whole plants, including leaves, stems and roots, were used.

Extraction and Isolation. Fresh plants (216 kg) were ground with MeOH and kept at room temperature for several weeks. After filtration, the filtrate was evaporated and partitioned successively with *n*-hexane, Et_2O, and EtOAc. The combined Et_2O and EtOAc extracts (170.2 g) were separated by Si gel column chromatography (EtOAc, EtOAc−EtOH, 97:3, 3:1, 1:1). The combined fraction (EtOAc-EtOH, 97:3 and 3:1, TLC guided, 45 g) was subjected to Si gel column chromatography (240−400 mesh, $CHCl_3$−MeOH, 98:2) and MPLC on ODS (Fuji gel, C_{18}, MeOH−H_2O, 1:1) to give portulene acetal (**6**, 425 mg).

Portulene Acetal (6): colorless oil; $[\alpha]^{25}_D$ +74.9° (*c* 0.25, MeOH); IR (film) ν_{max} 3400 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HREIMS (20 eV) $[M]^+$ (obsd *m*/*z* 366.2412, calcd 366.2406), $[M - OMe]^+$ (obsd *m*/*z* 335.2198, calcd 335.2222), $[M - MeOH]^+$ (obsd *m*/*z* 334.2140, calcd 334.2144).

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