Minor Sesquiterpenes with New Carbon Skeletons from the Brown Alga *Dictyopteris divaricata*

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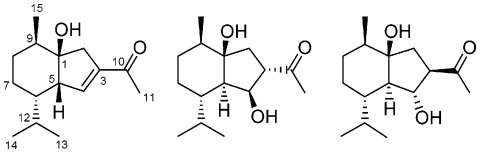
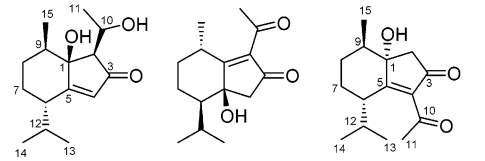
Five minor sesquiterpenes (**1**−**5**) with two novel carbon skeletons, together with a minor new oplopane sesquiterpene (**6**), have been isolated from the brown alga *Dictyopteris divaricata*. By means of spectroscopic data including IR, HRMS, 1D and 2D NMR, and CD, their structures including absolute configurations were assigned as (+)-(1*R*,5*S*,6*S*,9*R*)-3-acetyl-1-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-3-ene (**1**), (+)-(1*R*,3*S*,4*S*,5*R*,6*S*,9*R*)-3-acetyl-1,4-dihydroxy-6-isopropyl-9-methylbicyclo[4.3.0]nonane (**2**), (+)-(1*R*,3*R*,4*R*,5*R*,6*S*,9*R*)-3-acetyl-1,4-dihydroxy-6-isopropyl-9-methylbicyclo-[4.3.0]nonane (**3**), (+)-(1*S*,2*R*,6*S*,9*R*)-1-hydroxy-2-(1-hydroxyethyl)-6-isopropyl-9-methylbicyclo[4.3.0]non-4-en-3-one (**4**), (−)-(5*S*,6*R*,9*S*)-2-acetyl-5-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-1-en-3-one (**5**), and (−)-(1*S*,6*S*,9*R*)-4-acetyl-1-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-4-en-3-one (**6**). Biogenetically, the carbon skeletons of **1**−**6** may be derived from the co-occurring cadinane skeleton by different ring contraction rearrangements. Compounds **1**−**6** were inactive (IC_{50} > 10 μg/mL) against several human cancer cell lines.

Marine brown algae of the genus *Dictyopteris* have been reported to contain sesquiterpenoids[1−10] and C_{11} hydrocarbons.[11−13] As part of our program to systematically assess the chemical and biological diversity of seaweeds distributed in the gulf of the Yellow Sea, China,[14,15] we have reported cadinane sesquiterpenes[16] and norsesquiterpenes that are biogenetically derived from cadinane sesquiterpenes,[17] as well as other compounds,[18] from ethanolic extracts of *D. divaricata*. We report herein the isolation and structural elucidation of five minor components (**1**−**5**) belonging to two novel carbon skeletons and a minor new oplopane sesquiterpene (**6**). From a biogenetic point of view, the carbon skeletons of **1**−**6** may be derived from the co-occurring cadinanes by different ring contraction rearrangements, and these compounds may be the biogenetic intermediates of the co-occurring bisnorsesquiterpenes.[17]



1 **2** **3**



4 **5** **6**

Results and Discussion

The ethanolic extract of the alga was partitioned between water and ethyl acetate. The ethyl acetate phase was concentrated under vacuum and then subjected repeatedly to column chromatography over silica gel, Sephadex LH-20, and reversed-phase high-performance liquid chromatography (HPLC) to yield compounds **1**−**6**.

Compound **1** was obtained as a colorless gum with $[\alpha]_D^{20}$ +58 (*c* 0.11, MeOH) and showed absorption bands for hydroxy (3437 cm^{-1}) and conjugated carbonyl (1714, 1687, and 1612 cm^{-1}) functional groups in the IR spectrum. The EIMS of **1** gave a molecular ion peak at *m*/*z* 236 $[M]^+$, and the molecular formula was established as $C_{15}H_{24}O_2$ by HREIMS. The ^1H NMR spectrum in acetone-d_6 showed three methyl doublets at δ 0.97 (J = 7.0 Hz, CH_3-13), 0.91 (J = 6.5 Hz, CH_3-14), and 0.90 (J = 7.0 Hz, CH_3-15), one methyl singlet attributed to an acetyl group at δ 2.23 (CH_3-11), an olefinic proton multiplet at δ 6.88 (H-4), and an exchangeable hydroxy proton singlet at δ 3.63 (O*H*-1), as well as signals with complex coupling patterns attributed to methylene and methine protons (Table 1). The ^{13}C NMR and DEPT spectra of **1** showed 15 carbon signals for four methyls, three methylenes, five methines (one olefinic), and three quaternary carbons (one oxygen bearing, one olefinic, and one carbonyl). The protonated carbons and their bonded protons were unambiguously assigned by the HSQC experiment of **1** (Table 2). The $^1H-^1H$ COSY spectrum of **1** enabled extensive systems to be delineated. Vicinal and long-range coupling correlations from the olefinic proton (H-4) to a methine proton at δ 3.12 (1H, m, H-5) and a methylene proton at δ 2.24 (1H, dd, J = 15.5, 3.0 and 3.0 Hz, H-2α) indicated that the methine and methylene groups were attached to the two ends of the double bond, while correlations from a methine proton at δ 1.63 (1H, m, H-12) to the two methyls (H_3-13 and H_3-14) and another methine proton at δ 1.26 (1H, dddd, J = 12.5, 3.5, 3.0, and 2.0 Hz, H-6) indicated the presence of an isopropyl group attached to C-6. In addition, H-6 correlated with H-5 and H_2-7 at δ 0.85 (1H, dddd, J = 12.5, 12.5, 12.5, and 3.0 Hz, H-7α) and 1.79 (1H, dddd, J = 12.5, 3.5, 3.0, and 3.0, H-7β), and the coupling chain continued from H_2-7 through H_2-8 at δ 1.39 (1H, dddd, J = 12.5, 3.5, 3.5, and 3.5 Hz, H-8α) and 1.27 (1H, dddd, J = 12.5, 12.5, 12.5, and 3.0 Hz, H-8β) to H-9 at δ 1.42 (1H, m) and then to H_3-15. On the basis of the HMBC experiment of **1** the carbon skeleton and locations of functional groups were established for **1**. Two- and three-bond heteronuclear correlations (Figure 1) from H_2-2, H-4, and H_3-11 to both the carbonyl carbon at δ 196.2 (C-10) and the olefinic quaternary carbon at δ 145.0 (C-3) revealed that the acetyl group was located at C-3. Meanwhile, correlations from H_2-2, H-4, and H_3-15 to the quaternary carbon bearing oxygen at δ 82.9 (C-1) and from H_2-2 to both C-5 (δ 56.6) and C-9 (δ 35.4) demonstrated that C-1 was connected to C-2 (δ 42.9), C-5, and C-9 to form the

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Table 1. ^{1}H NMR Data for Compounds **1**−**6**[a]

no.	**1** (acetone-d_6)	**2** (acetone-d_6)	**2** ($CDCl_3$)	**3** (acetone-d_6)	**3** (methanol-d_4)	**4** (acetone-d_6)	**5** (acetone-d_6)	**6** (acetone-d_6)
2α	2.24 ddd (15.5,3.0,3.0)	1.63 dd (13.5,8.5)	1.72 dd (13.5, 8.0)	1.77 dd (13.5,11.5)	1.75 dd (14.0,11.5)			2.39 d (18.5)
2*β*	2.73 d (15.5)	2.23 dd (13.5,9.0)	2.11 dd (13.5,9.0)	1.96 dd (13.5, 2.0)	1.99 dd (14.0, 2.5)	2.21 d (2.5)		2.68 d (18.5)
3		3.22 dd (9.0,8.5)	3.27 dd (3.5,3.0)	2.96 ddd (11.5,6.0,2.0)	2.81 ddd (11.5,5.5,2.5)			
4α	6.88 m	4.32 dd (9.0,5.0)	4.27 d (5.5)			5.72 br s	2.95 d (18.5)	
4*β*				4.13 ddd (ca. 9.5,5.5,5.5)	4.16 dd (9.5,5.5)		2.35 d (18.5)	
5	3.12 m	1.09 dd (12.0,5.0)	1.14 dd (12.0,5.5)	1.38 dd (11.5,9.5)	1.34 dd (11.5,9.5)			
6	1.26 dddd (12.5, 3.5,3.0,2.0)	1.84 dddd (12.5,12.0,3.5,3.0)	1.73 ddd (12.5,12.0,3.5,3.0)	1.71 dddd (11.5,11.5,3.0,3.0)	1.61 dddd (12.5,11.5,3.0,3.0)	2.48 ddd (13.0,6.5,5.0)	2.04 ddd (ca. 13.5,6.5,5.0)	2.92 ddd (10.5,5.5,2.5)
7α	0.85 dddd (12.5,12.5,12.5,3.0)	1.02 dddd (12.5,12.5,12.0,4.0)	1.02 dddd (12.5,12.5,12.0,4.0)	0.95 dddd (12.5,12.5,12.5,4.0)	0.93 dddd (12.5,12.5,12.5,4.0)	1.12 dddd (13.0,13.0,13.0,3.5)	2.01 dddd (ca. 12.0,5.0,5.0,4.0)	1.84 dddd (14.0,4.0,4.0,2.5)
7*β*	1.79 dddd (12.5,3.5,3.0,3.0)	1.61 dddd (*ca.*12.5,3.5,3.0,3.0)	1.65 dddd (12.5,4.0,3.0,3.0)	1.55 dddd (11.5,4.0,3.0,3.0)	1.54 dddd (12.5,4.0,3.0,3.0)	2.00 ddd (13.0,4.0,3.5,3.5)	1.30 dddd (*ca.*13.5,12.5,12.0,4.0)	1.71 dddd (14.0,14.0,5.5,4.0)
8α	1.39 dddd (12.5,3.5,3.5,3.0)	1.44 dddd (12.5, 3.5, 3.5,3.5)	1.54 dddd (12.5,4.0,3.0,3.0)	1.37 dddd (ca. 12.5,4.0,3.0,3.0)	1.38 dddd (12.5,4.0,3.0,3.0)	1.53 dddd (13.0,4.0,3.5,3.5)	1.72 dddd (ca. 14.0,12.5,11.5,4.0)	2.42 dddd (14.0,14.0,4.0,4.0)
8*β*	1.27 dddd (12.5,12.5,12.5,3.0)	1.43 dddd (12.5,12.5,12.5,3.5)	1.27 dddd (13.0,12.5,12.5,4.0)	1.36 dddd (ca. 12.5,12.5,12.5,4.0)	1.25 dddd (12.5,12.5,12.5,4.0)	1.70 dddd (13.0, 13.0,13.0,3.5)	1.83 dddd (ca. 11.5,7.0,5.0,4.0)	1.23 dddd (14.0,4.0,4.0,2.5)
9	1.42 m	1.36 m	1.44 m	1.38 m	1.30 m	1.40 m	3.33 ddq (14.0,7.0,7.0)	2.32 m
10						4.25 m		
11	2.23 s	2.19 s	2.27 s	2.25 s	2.22 s	1.46 d (6.5)	2.34 s	2.34 s
12	1.63 m	2.02 m	1.98 m	2.19 m	2.13 m	1.93 m	1.94 m	2.52 m
13	0.97 d (7.0)	0.78 d (7.0)	0.77 d (7.0)	0.80 d (6.5)	0.77 d (7.0)	0.94 d (6.5)	0.72 d (6.5)	0.85 d (6.5)
14	0.91 d (6.5)	0.93 d (6.5)	0.93 d (7.5)	0.89 d (7.0)	0.88 d (7.0)	0.98 d (7.0)	0.98 d (7.0)	0.96 d (6.5)
15	0.90 d (7.0)	0.91 d (6.5)	0.91 d (6.5)	0.86 d (6.5)	0.81 d (6.5)	1.02 d (7.0)	1.33 d (7.0)	0.84 d (7.0)
1-OH	3.63 s	3.75s		2.82 s		4.31 s		4.50 s
4-OH		4.21 br d (8.5)		4.19 br d (5.5)				
5-OH							4.41 s	

[a] ^{1}H NMR data were measured at 500 MHz. Proton coupling constants (*J*) in Hz are given in parentheses. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

Table 2. ^{13}C NMR Data for Compounds **1**−**6**[a]

no.	1	2	3	4	5	6
1	82.9 qC	83.5 qC	80.4 qC	80.9 qC	189.8 qC	79.3 qC
2	42.9 CH_2	40.9 CH_2	37.9 CH_2	58.6 CH	138.0 qC	48.7 CH_2
3	145.0 qC	61.0 CH	60.0 CH	206.1 qC	203.5 qC	202.2 qC
4	144.3 CH	75.7 CH	80.4 CH	126.4 CH	48.0 CH_2	142.0 qC
5	56.6 CH	54.3 CH	58.5 CH	186.7 qC	79.3 qC	184.2 qC
6	42.4 CH	37.4 CH	41.9 CH	44.2 CH	50.6 CH	45.5 CH
7	28.7 CH_2	25.1 CH_2	25.3 CH_2	30.2 CH_2	20.3 CH_2	23.7 CH_2
8	31.0 CH_2	30.7 CH_2	30.8 CH_2	30.2 CH_2	29.3 CH_2	24.2 CH_2
9	35.4 CH	41.7 CH	41.6 CH	45.2 CH	32.9 CH	41.4 CH
10	196.2 qC	208.6 qC	212.7 qC	68.1 CH	197.8 qC	198.5 qC
11	25.7 CH_3	30.3 CH_3	29.3 CH_3	21.0 CH_3	31.0 CH_3	31.5 CH_3
12	31.3 CH	28.3 CH	28.1 CH	29.1 CH	29.5 CH	29.3 CH
13	21.2 CH_3	21.4 CH_3	16.3 CH_3	19.2 CH_3	18.4 CH_3	22.3 CH_3
14	21.7 CH_3	16.2 CH_3	21.8 CH_3	21.9 CH_3	23.4 CH_3	22.1 CH_3
15	15.6 CH_3	15.6 CH_3	15.3 CH_3	15.6 CH_3	21.7 CH_3	14.3 CH_3

[a] ^{13}C NMR data were measured in acetone-d_6 at 125 MHz. The assignments were based on DEPT, ^{1}H−^{1}H COSY, HMQC, and HMBC experiments.

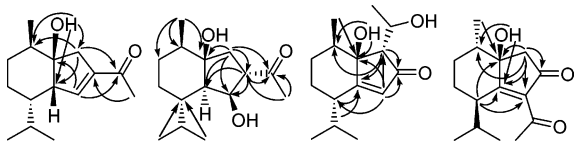

Figure 1. Key HMBC correlations of compounds **1**, **2**, **4**, and **6**.

planar structure 3-acetyl-1-hydroxy-6-isopropyl-9-methylbicyclo-[4.3.0]non-3-ene. In addition, the presence of the hydroxy group at C-1 was confirmed by HMBC correlations from the hydroxy proton to C-1, C-2, C-5, and C-9.

The relative stereochemistry of **1** was elucidated by an analysis of coupling constants (Table 1) and NOE difference experiments. In the ^{1}H NMR spectrum, coupling patterns of H-7α (dddd, $J_{6,7\alpha} = J_{7\alpha,7\beta} = J_{7\alpha,8\beta} = 12.5$ Hz, and $J_{7\alpha,8\alpha} = 3.0$ Hz) and H-8β (dddd, $J_{8\alpha,8\beta} = J_{7\alpha,8\beta} = J_{9,8\beta} = 12.5$ Hz, and $J_{7\beta,8\beta} = 3.0$ Hz) indicated characteristic *trans* diaxial relationships between H-6 (β) and H-7α and between H-8β and H-9 (α), suggesting equatorial orientations of both the isopropyl group at C-6 and the methyl at C-9. Meanwhile, the coupling pattern of H-6 (dddd, $J_{5,6\beta} = 2.0$ Hz, $J_{6\beta,7\beta} = 3.0$ Hz, $J_{6\beta,7\alpha} = 12.5$ Hz, and $J_{6\beta,12} = 3.5$ Hz) revealed an equatorial orientation (β) of H-5, which was supported by the splitting pattern of H-5 displayed as an unresolved narrow multiplet in the ^{1}H NMR spectrum of **1**. This was further confirmed by the NOE difference experiment of **1**, showing a strong NOE enhancement of H-6 by irradiation of H-5. The hydroxy group at C-1 must be in the axial orientation (β) so that the six-membered ring can fuse with the five-membered ring. From a biogenetic point of view, **1** may come from the co-occurring cadinane derivatives, and the absolute stereochemistry at C-1, C-6, and C-9 of **1** is therefore proposed to be identical to that of the co-occurring (−)-(1*R*,6S,7*S*,-10*R*)-1-hydroxycadinan-3-en-4-one, of which the absolute stereochemistry was determined by CD.[16] Therefore, the structure of **1** was assigned as (+)-(1*R*,5*S*,6*S*,9*R*)-3-acetyl-1-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-3-ene.

Compound **2** was obtained as a colorless gum with $[\alpha]_D^{20}$ +62 (*c* 0.10, MeOH) and showed IR absorption bands for hydroxy (3744 and 3419 cm^{-1}) and carbonyl (1707 cm^{-1}) groups. The positive FABMS gave a quasi-molecular ion peak at *m*/*z* 277 $[M + Na]^+$, and the molecular formula was determined as $C_{15}H_{26}O_3$ by HRFABMS. The NMR spectral data of **2** in Me_2CO-d_6 (Tables 1 and 2) were similar to those of **1** except that signals attributed to the double bond of **1** were absent in the NMR spectra of **2**, and instead, signals assignable to two methines appeared at δ_H 4.32 (1H, dd, $J = 9.0$ and 5.0 Hz, H-4) and 3.22 (1H, dd, $J = 9.0$ and 8.5 Hz, H-3) and δ_C 75.7 (C-4) and 61.0 (C-3), respectively. In addition, in the ^{1}H NMR spectrum of **2** there were two exchangeable hydroxy proton signals at δ 4.21 (brd, $J = 8.5$ Hz, O*H*-4) and 3.75 (s, O*H*-1). These data suggested that **2** was an analogue of **1** by addition of H_2O to the double bond, which was confirmed by ^{1}H−^{1}H COSY, HMQC, and HMBC experiments of **2** (Tables 1 and 2). The ^{1}H−^{1}H COSY spectrum of **2** showed consecutive vicinal coupling correlations from H-4 through H-5, H-6, H_2-7, and H_2-8 to H-9, and then to H_3-15, from H-6 through H-12 to both H_3-13 and H_3-14, and from H_2-2 to H-3. However, the vicinal correlation between H-3 and H-4 was not observed, indicating that the dihedral angle of the two vicinal protons was close to 90°. In the HMBC spectrum, long-range correlations (Figure 1) from H_2-2 and H_3-15 to C-1 and from H_2-2 and H-3 to C-4 and C-5 confirmed the presence of the bicyclo[4.3.0]nonane ring system in **2**. In addition, the HMBC correlations of C-10 with H_2-2, H-3, and H_3-11 and of C-3 with H_2-2 and H_3-11 demonstrated the location of the acetyl at C-3, while correlations of C-6 with H_3-13 and H_3-14 and of C-1, C-8, and C-9 with H_3-15 confirmed the locations of the isopropyl at C-6 and the methyl at C-9.

The relative configuration of **2** was determined by an analysis of coupling constants and NOE difference experiments. In the ^{1}H NMR spectrum of **2** in $CDCl_3$, the signal of the hydroxy proton was completely exchanged by the deuteriated solvents, and H-4 appeared clearly as a doublet with a coupling constant of 5.5 Hz, while H-5 was a double doublet with coupling constants of 12.0 and 5.5 Hz. These coupling patterns unambiguously revealed a *trans*-diaxial relationship between H-5 and H-6 ($J_{5,6} = 12.0$ Hz). Furthermore, the coupling patterns of H-6, H_2-7, H_2-8, and H-9 (Table 1) indicated that the six-membered ring possessed a chair conformation and that the coupling constant of 12.5 Hz between H-8β and H-9 established the axial orientation of H-9. Although the ^{1}H NMR spectrum of **2** in $CDCl_3$ gave better resolution, **2** was found to be unstable in $CDCl_3$ due to the possible existence of acid in the solvent. In NOE difference experiments of **2** in acetone-d_6, strong enhancements of H-5, H-12, H_3-11, and H_3-14 were observed by irradiation of H-4, while H-2β and the exchangeable protons of the two hydroxys at C-1 and C-4 were enhanced by irradiation of H-3. In addition, irradiation of H-2β gave enhancements of H-3, H_3-15, and the exchangeable proton of the hydroxy at C-1. These data demonstrated that **2** possessed a *trans*-junction ring system, that H-4 and H-5, together with the acetyl and the isopropyl, were on the same side of the ring system, and that H-3, H-2β, and H_3-15, as well as the two hydroxy groups, were on the other side of the ring system. In consideration of the similar biogenetic origin of **1** and **2**, the absolute configurations at C-6 and C-9 of **2** were proposed as 6*S* and 9*R*, respectively. Therefore, the structure of **2** was assigned as (+)-(1*R*,3*S*,4*S*,5*R*,6*S*,9*R*)-3-acetyl-1,4-dihydroxy-6-isopropyl-9-methylbicyclo[4.3.0]nonane.

Compound **3** was obtained as a colorless gum with $[\alpha]_D^{20}$ +55 (*c* 0.40, MeOH) and showed very similar IR, MS, and NMR spectral features to those of **2**. The ^{1}H−^{1}H COSY, HMQC, and HMBC data analyses of **3** revealed that **3** had an identical planar structure to **2** and led to unambiguous assignments of the NMR spectroscopic data for **3** (Tables 1 and 2). By comparing the NMR data of **2** and **3**, chemical shift values and coupling patterns of the five-membered ring moiety of **3** were obviously different from those of **2**, indicating that the difference between **3** and **2** involved the stereochemistry of the five-membered ring moiety. The NMR spectra of **3** were obtained in both acetone-d_6 and methanol-d_4 (Table 1 and 2). The ^{1}H NMR spectrum of **3** in methanol-d_4 possessed better resolution, which readily distinguished the coupling patterns of all protons. The coupling patterns of the five-membered ring moiety including H_2-2 at δ 1.75 (1H, dd, $J = 14.0$ and 11.5 Hz, H-2α) and 1.99 (1H, dd, $J = 14.0$ and 3.0 Hz, H-2β), H-3 at δ 2.81 (1H, ddd, $J = 11.5$, 5.5, and 3.0 Hz), H-4 at δ 4.16 (1H, dd, $J = 9.5$ and 5.5 Hz), and H-5 at δ 1.34 (1H, dd, $J = 11.5$ and 9.5 Hz) indicated that the coupling constants of H-3 with H-2α, H-2β, and H-4 in **3** were 11.5, 3.0, and 5.5 Hz, respectively, instead of 8.0, 9.0, and 0.0 Hz

in **2**, indicating that the configuration at C-3 of **3** differed from that of **2**. In addition, the coupling constant between H-4 and H-5 (9.5 Hz) in **3** was larger than that (5.0 Hz) in **2**, strongly suggesting that the configuration at C-4 of **3** was also different from that of **2**. These indications were supported by the NOE difference experiment of **3**, which showed enhancements of H-5 and H-2α by irradiation of H-3 and of H-6 by irradiation of H-4. Therefore, the structure of **3** was determined as (+)-(1*R*,3*R*,4*R*,5*R*,6*S*,9*R*)-3-acetyl-1,4-dihydroxy-6-isopropyl-9-methylbicyclo[4.3.0]nonane.

Compound **4** was obtained as a colorless gum with $[\alpha]_D^{20}$ +51 (*c* 0.10, MeOH) and showed absorption bands for hydroxy (3415 cm^{-1}), conjugated carbonyl (1682 cm^{-1}), and double bond (1612 cm^{-1}) groups. The positive FABMS exhibited a quasi-molecular ion peak at *m*/*z* 253 $[M + H]^+$, and the HRFABMS at *m*/*z* 253.1809 $[M + H]^+$ established the molecular formula $C_{15}H_{24}O_3$. The ^1H NMR spectrum (Me_2CO-d_6) showed four methyl doublets at δ 0.94 (J = 6.5 Hz, CH_3-13), 0.98 (J = 7.0 Hz, CH_3-14), 1.02 (J = 7.0 Hz, CH_3-15), and 1.46 (J = 6.5 Hz, CH_3-11), an oxymethine multiplet at δ 4.25 (H-10), and a broad singlet attributed to an olefinic proton at δ 5.72 (H-4). The ^{13}C NMR and DEPT spectra showed 15 carbon signals (Table 2), of which one signal attributed to C-1 at δ 80.9 did not clearly appear due to limited acquisition time and sample amount but was confirmed by HMBC experiment (see Supporting Information), indicating that **4** is another sesquiterpene with functional groups of one carbonyl, two hydroxyls, and one trisubstituted double bond. The 2D NMR spectroscopic data analysis of **4** revealed that it possessed another new carbon skeleton different from that of **1**−**3**. On the basis of the HSQC experiment, signals of protons and their corresponding carbons were unambiguously assigned (Table 1 and 2). In the $^1H-^1H$ COSY spectrum the spin system in a linear sequence were traced from H-6 through H_2-7 and H_2-8 to H-9 and terminating at H_3-15 in one end and through H-12 to H_3-13 and H_3-14 in another end, demonstrating the presence of the structural unit $CH_3CHCH_2CH_2CHCH(CH_3)_2$. Meanwhile, homonuclear correlations of H-10 with H_3-11 and H-2 revealed the presence of a spin coupling unit, $CH_3CH(OH)CH$. In the HMBC spectrum, two- and three-bond heteronuclear correlations (Figure 1) from H_3-15 to C-1 (δ 80.9), from H-2 to C-9 (δ 45.2), and from the exchangeable hydroxy proton at δ 4.31 (1H, s) to both C-1 and C-9 unequivocally established the direct connections of C-1 with C-2 (δ 58.6) and C-9, while correlations from H-2, H-6, and H-12 to the olefinic quaternary carbon at δ 186.7 (C-5) and from the olefinic proton (H-4) to C-1 and C-6 (δ 44.2) demonstrated the connections of C-5 with C-1 and C-6 to form the six-membered ring. In addition, correlations from H-4 to C-2 and C-3 and from H-2 to C-3 disclosed the five-membered enone ring. The deshielded chemical shift value of C-5 further confirmed the conjugation between the carbonyl and the double bond of the five-membered ring.[19] Therefore, the planar structure of **4** was elucidated to be 1-hydroxy-2-(1-hydroxyethyl)-6-isopropyl-9-methylbicyclo-[4.3.0]non-4-en-3-one, which possesses an unusual carbon skeleton.

The relative stereochemistry of **4** was deduced from the coupling constant analysis and NOE difference experiments of **4**. The ^1H NMR spectrum of **4** showed that the coupling constants between H-6 and H-7α and between H-8β and H-9 are 13.0 Hz (Table 1), indicating that both the isopropyl at C-6 and the methyl at C-9 were equatorial. In the NOE difference experiments of **4** H-7β, H-8β, and the proton of the hydroxy at C-1 were enhanced by irradiation of H-6, indicating an axial (β) orientation of the hydroxy at C-1. An enhancement of H-9 by irradiation of H-2 suggested an α-orientation of H-2. The absolute configuration of **4** was determined by a combination of the CD spectrum (see Supporting Information Figure a) and the biogenetic origin of **4** from the co-occurring cadinane derivatives.[16,17] The molecular modeling using the MM2 program indicated that the torsion angle between the double bond and the ketone group is 174.5° for the lowest energy conformation of **4**. These demonstrated that the enone moiety of **4**

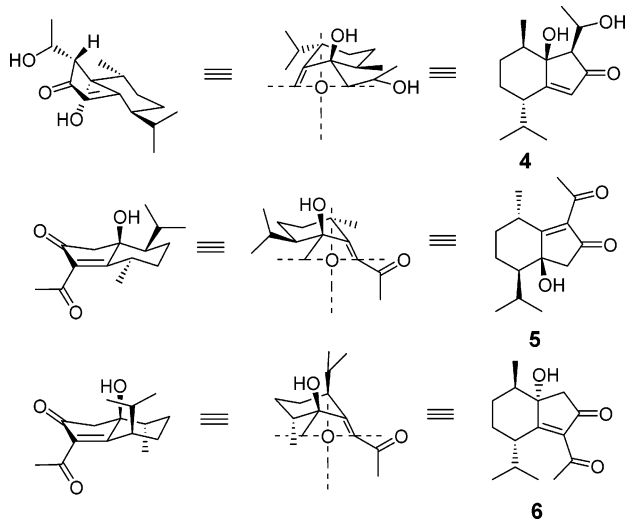

Figure 2. Configurations of compounds **4**−**6**.

was not planar. On the basis of the octant rule for the cyclopentenone,[20] the positive Cotton effect at 321 nm ($\Delta\epsilon_{max}$ +4.38) for n→π* and the negative Cotton effect at 233 nm ($\Delta\epsilon_{max}$ −13.16) for π→π* indicated that the conformation of **4** is as depicted in Figure 2. Therefore, the structure of **4** was determined as (+)-(1*S*,2*R*,6*S*,9*R*)-1-hydroxy-2-(1-hydroxyethyl)-6-isopropyl-9-methylbicyclo[4.3.0]non-4-en-3-one. The configuration at C-10 has not been determined yet.

Compound **5** was obtained as a colorless gum with $[\alpha]_D^{20}$ −80 (*c* 0.10, MeOH). The IR, EIMS, and NMR spectroscopic data indicated that **5** is an analogue of **4**, which was confirmed by the 2D NMR data analysis of **5**. The $^1H-^1H$ COSY spectrum of **5** indicated that the linear spin system of the six-membered ring moiety of **5** was completely identical to that of **4**. However, a comparison of the NMR data of **5** and **4** (Tables 1 and 2) indicated that the chemical shift values of H-9 and H_3-15 of **5** were significantly deshielded by Δδ 1.93 and 0.31 ppm, respectively, suggesting the location of the double bond between C-1 and C-2 in **5** instead of between C-4 and C-5 in **4**. This was confirmed by the appearance of two quaternary carbon signals assignable to a tetrasubstituted double bond at δ 189.8 (C-1) and 138.0 (C-2) in the ^{13}C NMR spectrum of **5**. In the ^1H NMR spectrum of **5** the disappearance of the olefinic proton signal and the appearance of signals attributed to the characteristic AB system of an isolated methylene at δ 2.95 (1H, d, J = 18.5 Hz, H-4α) and 2.35 (1H, d, J = 18.5 Hz, H-4β), as well as the chemical shift of H-6 (δ 2.04), indicated that the hydroxy was at C-5 in **5** rather than at C-1 in **4**. Meanwhile, in the ^1H and ^{13}C NMR spectra of **5** the disappearance of the oxymethine proton and carbon signals and the appearance of the carbonyl carbon at δ 197.8 demonstrated the replacement of the oxymethine (C-10) of **4** by the carbonyl group of **5**, which was supported by the replacement of the methyl doublet (CH_3-11) of **4** by the methyl singlet at δ 2.43 in the ^1H NMR spectrum of **5**. The coupling constants between H-6 and H-7β ($J_{6,7\beta}$ = 13.5 Hz) and between H-9 and H-8α ($J_{8\alpha,9}$ = 14.0 Hz) indicated equatorial orientations for both the isopropyl at C-6 and the methyl at C-9. In the NOE difference spectrum, irradiation of the hydroxy proton gave enhancements of H-9, H_3-11, H_3-14, and H_3-15, indicating that they are oriented on the same side of the ring system. The molecular modeling using the MM2 program indicated that the torsion angle between the double bond and the ketone group is −175.1° for the lowest energy conformation of **5**. In the CD spectrum (see Supporting Information Figure b), a negative Coton effect at 320.5 nm ($\Delta\epsilon_{max}$ −6.30) for n→π* and the positive Cotton effect at 252 nm ($\Delta\epsilon_{max}$ +2.94) for π→π* suggested that the configuration of **5** is as depicted in Figure 2. Accordingly, the structure of **5** was

determined as (−)-(5*S*,6*R*,9*S*)-2-acetyl-5-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-1-en-3-one.

Compound **6** was obtained as a colorless gum with $[\alpha]_D^{20}$ −16 (*c* 0.10, MeOH). The IR and EIMS spectroscopic data were very similar to those of **5**, while the HREIMS at *m*/*z* 250.1579 $[M]^+$ confirmed that it was an isomer of **5**. However, chemical shift values and coupling patterns of protons in the NMR spectrum of **6** (Tables 1 and 2) were significantly different from those of **5**. The proton and protonated carbon signals of **6** were carefully assigned by the $^1H-^1H$ COSY and HSQC experiments (Table 1 and 2), and the $^1H-^1H$ COSY spectroscopic data of **6** confirmed the presence of the linear spin coupling of the six-membered ring moiety identical to that of **5**. In the HMBC spectrum of **6**, correlations (Figure 1) from H_3-15 to C-1 and from the hydroxy proton to C-1, C-5, and C-9, together with the chemical shift value of these carbons, established unambiguously the location of the hydroxy group at C-1, while correlations from H-6 to C-1, C-4, and C-5 in combination with the chemical shift values of C-4 and C-5 revealed that the double bond was located between C-4 and C-5. In addition, two- and three-bond HMBC correlations from H_2-2 to C-1, C-3, C-4, C-5, and C-9 confirmed unequivocally that one carbonyl carbon (C-3) connected with C-2 and C-4 to form the unsaturated five-membered ring, while the HMBC correlation from H_3-11 to C-4 indicated that the acetyl group was located at C-4. This deduction revealed that compound **6** possesses the oplopane skeleton,[21] and therefore, the planar structure of **6** is 4-acetyl-1-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-4-en-3-one.

In the ^1H NMR spectrum of **6**, coupling patterns of H-6 and H_2-7 (Table 1) showed that the coupling constants of H-6 with H-7α and H-7*β* were 2.5 and 5.5 Hz, respectively. This indicated an axial orientation of the isopropyl at C-6. Meanwhile, the coupling pattern of H_2-8 indicated that the coupling constants of H-9 with H-8α and H-8*β* are 4.0 and 2.5 Hz, respectively, indicating that the methyl at C-9 is axial also. In the NOE difference experiment, irradiation of the hydroxy proton enhanced H-2α, and irradiation of H-2*β* gave an enhancement of H_3-15, demonstrating an α-orientation of the hydroxy at C-1. With a torsion angle of 177.1° the molecular modeling using the MM2 program indicated that the double bond and the ketone group of the five-membered ring is not planar for the lowest energy conformation of **6**. The CD spectrum (see Supporting Information Figure c) of **6** gave a negative Cotton effect at 321 nm ($\Delta\epsilon_{max}$ −7.69) for n→*π** and the positive Cotton effect at 253 nm ($\Delta\epsilon_{max}$ +1.99) for *π*→*π**, suggesting that the absolute configuration of **6** is as depicted in Figure 2. Therefore, the structure of **6** was determined as (+)-(1*S*,6*S*,9*R*)-4-acetyl-1-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-4-en-3-one.

From the biogenetic point of view, the three carbon skeletons of **1**−**6** are derived from the co-occurring cadinane skeleton by different ring contraction rearrangements, and these minor components may be the biogenetic intermediates of the co-occurring bisnorsesquiterpenes.[17]

Compounds **1**−**6** were tested for cytotoxicity against lung adnocarcinoma (A549), hepatoma (Bel7402), stomach cancer (BGC-823), colon cancer (HCT-8), and breast cancer (MCF-7) cell lines by using the MTT method,[22,23] but were found to be inactive (IC_{50} > 10 *μ*g/mL).

Experimental Section

General Experimental Procedures. Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. Optical rotations were measured on a Rudolph Research Autopol III automatic polarimeter. The CD spectrum was recorded on a Jasco J-715 spectropolarimeter. IR spectra were recorded as KBr disks on a Nicolet Impact 400 FT-IR spectrophotometer. 1D- and 2D-NMR spectra were obtained at 500 and 125 MHz for ^1H and ^{13}C, respectively, on an Inova 500 MHz spectrometer in acetone-d_6 or $CDCl_3$ or MeOH-d_4 with solvent peaks as references. EIMS, FABMS, HREIMS, and HRFABMS data were measured with a Micromass Autospec-Ultima ETOF spectrometer. Column chromatography was performed with Si gel (200−300 mesh) and Sephadex LH-20. TLC was carried out with glass precoated Si gel GF_{254} plates. Spots were visualized under UV light or by spraying with 7% H_2SO_4 in EtOH followed by heating. HPLC was performed using an Alltima C18 10 *μ*m preparative column (22 × 250 mm).

Plant Material. As described in a previous report.[17]

Extraction and Isolation. The preliminary separation procedure has been described in a previous report.[17] The EtOAc fraction (120.3 g) was chromatographed over Si gel (1200 g) eluting with a gradient of increasing Me_2CO (0−100%) in petroleum ether. The resulting fractions designated I−XXIV were based upon TLC analyses. Fractions XI, XIII, and XIV were separated by CC over Sephadex LH-20 using petroleum ether−$CHCl_3$−MeOH (5:5:1) as the eluent to give corresponding subfractions. The second subfraction of XI was purified by reversed-phase preparative HPLC using MeOH−H_2O (80:20) to give compound **1** (5.2 mg). The third subfraction of XI was purified by reversed-phase preparative HPLC using MeOH−H_2O (75:25) to yield compounds **4** (2.1 mg), **5** (4.0 mg), and **6** (16.6 mg). The second subfraction of XIII was purified by reversed-phase preparative HPLC using MeOH−H_2O (80:20) to yield compound **3** (6.0 mg). The second subfraction of XIV was purified by reversed-phase preparative HPLC using MeOH−H_2O (80:20) to yield compound **2** (4.8 mg).

(+)-(1*R*,5*S*,6*S*,9*R*)-3-Acetyl-1-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-3-ene (1): colorless gum; $[\alpha]_D^{20}$ +58 (*c* 0.11, MeOH); IR (KBr) ν_{max} 3437, 2958, 2933, 2873, 1714, 1687, 1612, 1456, 1369, 1265, 1144, 991, 883, 860, 746 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz), see Tables 1 and 2; EIMS *m*/*z* (%) 236 (25) $[M]^+$, 221 (6), 209 (4), 193 (100), 175 (15), 165 (13), 151 (20), 137 (12), 123 (10), 109 (13), 95 (7), 69 (7), 58 (16), 55 (11), 43 (82); HREIMS *m*/*z* 236.1778 (calcd for $C_{15}H_{24}O_2$, 236.1776).

(+)-(1*R*,3*S*,4*S*,5*R*,6*S*,9*R*)-3-Acetyl-1,4-dihydroxy-6-isopropyl-9-methylbicyclo[4.3.0]nonane (2): colorless gum; $[\alpha]_D^{20}$ +62 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3477, 3419, 2962, 2929, 2873, 1707, 1419, 1358, 1215, 1178, 1022, 978, 877 cm^{-1}; ^1H NMR (acetone-d_6 and $CDCl_3$, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz), see Tables 1 and 2; FABMS *m*/*z* (%) 277 (65) $[M + Na]^+$, 237 (10) $[M + H - H_2O]^+$, 219 (100) $[M + H - 2H_2O]^+$; HRFABMS *m*/*z* 277.1754 (calcd for $C_{15}H_{26}O_3Na$, 277.1780).

(+)-(1*R*,3*R*,4*R*,5*R*,6*S*,9*R*)-3-Acetyl-1,4-dihydroxy-6-isopropyl-9-methylbicyclo[4.3.0]nonane (3): colorless gum; $[\alpha]_D^{20}$ +55 (*c* 0.40, MeOH); IR (KBr) ν_{max} 3425, 2960, 2933, 2871, 1685, 1464, 1417, 1365, 1340, 1178, 1153, 1036, 1061, 1005, 976, 928, 903, 874, 796 cm^{-1}; ^1H NMR (acetone-d_6 and MeOH-d_4, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz), see Tables 1 and 2; EIMS *m*/*z* (%) 254 (20) $[M]^+$, 236 (65) $[[M - H_2O]^+$, 226 (13) $[M - CO]^+$, 218 (22), 211 (31), 193 (97), 183 (76), 175 (45), 165 (19), 149 (17), 137 (21), 111 (23), 71 (21), 58 (24), 43 (100); HREIMS *m*/*z* 254.1871 (calcd for $C_{15}H_{26}O_3$, 254.1882).

(+)-(1*S*,2*R*,6*S*,9*R*)-1-Hydroxy-2-(1-hydroxyethyl)-6-isopropyl-9-methylbicyclo[4.3.0]non-4-en-3-one (4): colorless gum; $[\alpha]_D^{20}$ +51 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3415, 2960, 2929, 2871, 1682, 1612, 1450, 1388, 1238, 1165, 1117, 1036, 989, 822 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz), see Tables 1 and 2; positive FABMS *m*/*z* 253 (60) $[M + H]^+$, 235 (100) $[M + H - H_2O]^+$, 217 (45) $[M + H - 2H_2O]^+$; HRFABMS *m*/*z* 253.1809 $[M + H]^+$ (calcd for $C_{15}H_{25}O_3$ 253.1804).

(−)-(5*S*,6*R*,9*S*)-2-Acetyl-5-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-1-en-3-one (5): colorless gum; $[\alpha]_D^{20}$ −80 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3431, 2960, 2937, 2873, 1711, 1693, 1606, 1464, 1361, 1298, 1176, 1018 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz), see Tables 1 and 2; EIMS *m*/*z* (%) 250 (12) $[M]^+$, 235 (4) $[M - CH_3]^+$, 223 (8) 207 (6), 205 (6), 189 (46), 179 (5), 163 (11), 147 (6), 69 (11), 58 (15), 43 (100); HREIMS *m*/*z* 250.1579 $[M]^+$ (calcd for $C_{15}H_{22}O_3$ 250.1569).

(-)-(1*S*,6*S*,9*R*)-4-Acetyl-1-hydroxy-6-isopropyl-9-methylbicyclo[4.3.0]non-4-en-3-one (6): colorless gum; $[\alpha]_D^{20}$ −16 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3450, 2964, 2871, 1709, 1693, 1606, 1466, 1385, 1361, 1313, 1232, 1186, 1155, 1020, 895 cm^{-1}; ^1H NMR (acetone-d_6, 500 MHz) and ^{13}C NMR (acetone-d_6, 125 MHz), see Tables 1 and 2; EIMS *m*/*z* (%) 250 (60) $[M]^+$, 232 (68) $[M - H_2O]^+$, 217 (100) $[M - H_2O - CH_3]^+$, 207 (52), 199 (12), 190 (35), 189 (33), 186 (30), 175 (78), 171 (22), 165 (21), 147 (12), 123 (10), 111 (15), 95 (12), 69 (6), 55 (8), 43 (83); HREIMS *m*/*z*250.1577 $[M]^+$ (calcd for $C_{15}H_{22}O_3$ 250.1569).

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Supporting Information Available: MS and ^{1}H and ^{13}C NMR spectra of compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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