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Corymbulosins D–H, 2-Hydroxy- and 2-Oxo-clerodane Diterpenes from the Bark of *Laetia corymbulosa*

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Abstract

A bioactive CH₃OH–CH₂Cl₂ (1:1) extract of the bark of *Laetia corymbulosa* provided five new clerodane diterpenes with an isozuelanin skeleton, designated as corymbulosins D–H (**1–5**), as well as the known corymbulosins B (**6**) and C (**7**), for which the relative configurations were not previously determined. The structures of **1–5** were characterized on the basis of 1D and 2D NMR spectroscopic and HRMS analysis. The absolute configurations of all isolated compounds **1–7** were verified through chemical methods, including modified Mosher esterifications or oxidation of the hydroxy group at C-2, ECD experiments, and spectroscopic data comparison. The isolated compounds were evaluated for antiproliferative activity against a small panel of human cancer cell lines.

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ASSOCIATED CONTENT

Supporting Information

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Various 1D- and 2D-NMR spectra for **1–7**, ¹H NMR spectra for the Mosher esters of **1–3**, **6**, and **7**, ¹H/¹³C NMR spectra of the product after oxidation of **7**, as well as NCI-60 human tumor cell line assay data for the crude organic extract of *L. corymbulosa* (N005829) (PDF)

The authors declare no competing financial interest.

Graphical Abstract

The genus *Laetia* contains about 35 species distributed in tropical areas of South America, including Brazil, Colombia, and Peru.¹ This genus belongs to the family Salicaceae, and it was formerly placed in the Flacourtiaceae, which is known as a source of isozuelanin-type tricyclic clerodane diterpenes.² Numerous analogues have been generated with diverse substituent patterns at C-2, C-6, C-7, C-18, and C-19, as well as many stereoisomeric combinations; however, the absolute stereochemistry was not determined in most cases. A known major biological activity of isozuelanin-type clerodanes is potent cytotoxicity. In recent years, studies have reported that these compounds also act as NGF potentiators,³ reticulum Ca^{2+} -ATPase inhibitors,⁴ and TRAIL sensitizers.⁵ So far, little phytochemical research on the genus *Laetia* has been reported,^{6–8} and in particular, only one study on *Laetia corymbulosa* was published in 2000.⁹

In a continuing investigation on bioactive compounds from rainforest plants,¹⁰ *L. corymbulosa* was selected for exploration of its cytotoxic components. A $\text{CH}_3\text{OH}-\text{CH}_2\text{Cl}_2$ (1:1) extract of the bark of this species (N005829), as provided by the U.S. National Cancer Institute (NCI, Frederick, MD, USA), was found to exhibit broad cytotoxicity in the NCI-60 human tumor cell assay ($\text{IC}_{50} < 10 \mu\text{g/mL}$ against all 60 cell lines, as performed by NCI in 1989). The bioactivity-directed fractionation and isolation of N005829 based on cytotoxicity yielded five new tricyclic clerodane diterpenes, designated corymbulosins D–H (**1–5**), and two known corymbulosins, B (**6**) and C (**7**).⁶

RESULTS AND DISCUSSION

The EtOAc-soluble portion of the $\text{CH}_3\text{OH}-\text{CH}_2\text{Cl}_2$ (1:1) extract of *L. corymbulosa* (bark) was separated by a combination of column chromatography, preparative HPLC, and preparative TLC methods, using silica gel and octadecylsilane (ODS). The isolated new diterpenes **1–5** were characterized by 1D and 2D NMR spectroscopic and HRMS analysis.

Compound **1** was obtained as an optically active colorless oil, $[\alpha]_{\text{D}}^{25} - 6.4$ (c 0.27, CHCl_3). The molecular formula, $\text{C}_{32}\text{H}_{48}\text{O}_8$, was determined by HRFABMS, from the peak at m/z 583.3295 $[\text{M} + \text{Na}]^+$. The IR absorption bands at 3497, 1761, and 1738 cm^{-1} suggested the presence of hydroxy and ester carbonyl groups, respectively. In the NMR spectra, two singlet peaks at δ_{H} 1.90 and 2.07 (Table 1) together with two peaks around δ_{C} 170 and 21 (Table 2) implied the presence of two acetoxy groups. Other proton signals were attributable to two methyls [δ_{H} 0.92 (3H, d, $J = 6.8 \text{ Hz}$), 0.99 (3H, s)], two vinyl protons [δ_{H} 6.00 (1H, m), 6.43 (1H, dd, $J = 17.4, 10.8 \text{ Hz}$)], four methylenes protons [δ_{H} 5.22 (1H, d, $J = 17.4$

Hz), 5.04 (1H, d, J = 10.8 Hz), 5.05 (1H, s), and 4.94 (1H, s)], and two acetal-acyloxy methine protons [δ_{H} 6.50 (1H, m), 6.54 (1H, s)]. These signals were consistent with the basic skeleton of known tricyclic clerodane diterpenes isolated from genus *Laetia*. In addition, two methylene protons at δ_{H} 2.31 (m), three methyl protons at δ_{H} 0.87 (t, J = 7.2 Hz), and ten methylene protons at δ_{H} 1.28 (m) and 1.61 (m) suggested that an octanoyloxy group is attached to the core skeleton. The ^{13}C NMR spectrum of **1** (Table 2) showed 32 signals, including those for three ester carbonyls (δ_{C} 173.3, 170.1, 170.0), three pairs of olefinic carbons (δ_{C} 142.6 and 126.4, 145.2 and 140.3, 115.4 and 112.6), a hydroxylated carbon (δ_{C} 63.8), an esterified carbon (δ_{C} 73.7), and two acetal carbons (δ_{C} 98.3, 95.4). These carbon signals were assigned to C-1', OAc-18, OAc-19, C-4, C-3, C-13, C-14, C-15, C-16, C-2, C-6, C-19, and C-18, respectively, through ^1H - ^1H COSY and HMBC experiments (Figure 1). The locations of three acyl groups were also determined by HMBC (Figure 1). The correlations between H-6 and C-1', as well as H-18/H-19 and the acetyl carbonyls, supported that the octanoyloxy group was located at C-6 and two acetyl groups were located at C-18 and C-19, respectively. The relative configuration of **1** was determined from the NOESY data. The *s-trans* conformation of the diene side chain was supported by the NOESY correlation between H-15 and H-12. Other NOESY correlations, such as between H-6 and H-8, H-2' and H-18, H-10 and H-12, as well as H_{ax} -7 and H-19, together with the above-mentioned spectroscopic data, reinforced the postulation that compound **1** is an analogue of corymbulosin B⁶ with the same relative configuration but with an octanoyloxy rather than a decanoyloxy group at C-6. To determine the absolute configuration, the OH group at C-2 was converted to *R*- and *S*-Mosher esters. On the basis of the δ_{H} (*S*-*R*) values of the ^1H NMR signals, the absolute configuration of C-2 was detected as the *R* form, as shown in Figure 2. Thus, the structure of **1** was established as (2*R*, 5*S*, 6*S*, 8*R*, 9*R*, 10*S*, 18*R*, 19*S*)-18,19-di-*O*-acetyl-18,19-epoxy-2-hydroxy-6-octanoyloxycyclo-3,13(16),14-triene and given the trivial name corymbulosin D, following the related tricyclic clerodane diterpenes corymbulosins A–C⁹ from *L. corymbulosa*. The experimental electronic circular dichroism (ECD) data are shown in Figure 3.

The assigned molecular formula of **2** was $\text{C}_{34}\text{H}_{48}\text{O}_8$ from the observation of the HRFABMS ion peak at m/z 607.3221 [$\text{M} + \text{Na}$]⁺. According to the ^1H and ^{13}C NMR spectra (Tables 1 and 2), the same basic tricyclic clerodane skeleton was found in **1** and **2**. However, four sp^2 carbons at δ_{C} 143.5, 125.6, 141.7, and 117.5 in the ^{13}C NMR spectrum of **2** suggested the presence of two additional double bonds. ^1H - ^1H COSY and HMBC experiments indicated that these double bonds are contiguous to form a diene and are also connected to C-1' (Figure 1). The coupling constants (11.4 Hz) of the doublet at C-2' as well as the NOESY correlation between C-3' and C-6' suggested *cis* forms for both olefins. These observations led to the conclusion that compound **2** is an analogue of corymbulosin D (**1**) with a (2'*Z*, 4'*Z*)-2',4'-decadienoate substituent replacing the octanoate at C-6. The same relative configuration was revealed by NOESY correlations to those as mentioned above for corymbulosin D (**1**). The absolute configuration was further determined by the modified Mosher ester method. The analysis of δ_{H} (*S*-*R*) values allowed the absolute configuration of C-2 to be defined as *R* (Figure 2). Thus, this compound (corymbulosin E) was characterized as (2*R*, 5*S*, 6*S*, 8*R*, 9*R*, 10*S*, 18*R*, 19*S*)-6-[(2*Z*, 4*Z*)-deca-2,4-dienoyl]-oxy-18,19-di-*O*-acetyl-18,19-epoxy-2-hydroxycyclo-3,13(16),14-triene. The experimental ECD data

also confirmed that compounds **2** and **1** have the same absolute configuration (Figure 3), although slight differences were found in the curves due to the presence of the conjugated double bond in the side chain of **2**.

Compound **3** displayed a Na⁺ adduct molecular-related ion at m/z 607.3213 in the HRFABMS, corresponding to the same molecular formula, C₃₄H₄₈O₈, as **2**. However, differences were observed in their optical rotation values, with $[\alpha]^{25}_D +23.3$ (c 0.12, CHCl₃) for **2** and -31.1 (c 0.30, CHCl₃) for **3**. The ¹H and ¹³C NMR spectra (Tables 1 and 2) of **3** were closely comparable with those of **2**, except for the C-2 signals (δ_c 69.2 for **2** and δ_c 64.3 for **3**), which suggested an opposite configuration at this position. The NOESY data of **3** resembled those of **2** with the exception of a correlation between H-2 and H-10 that was observed only in **2** (Figure 1). These results indicated that compound **3** is the C-2 epimer of **2**, and this assignment was strongly supported by using the modified Mosher ester method (Figure 2). The experimental ECD data also verified the absolute configuration of **3** to be different from those of **1** and **2** (Figure 3). Thus, compound **3** (corymbulosin F) was defined as (2*S*,5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-6-[(2*Z*,4*Z*)-deca-2,4-dienoyl]oxy-18,19-di-*O*-acetyl-18,19-epoxy-2-hydroxycyclocleroda-3,13(16),14-triene.

HRFABMS of compound **4** showed a molecular formula of C₃₆H₅₆O₈ with a Na⁺ adduct molecular-related ion at m/z 639.3873. The ¹H NMR spectrum of **4** (Tables 1) was identical to that of the known compound, corymbulosin C (**7**), except for the integrated ratio of aliphatic protons around δ_H 1.20–1.35. In addition, comparison of the 1D NMR spectra of **4** and **7** indicated four additional aliphatic protons as well as two additional carbons. These data implied that compound **4** has a dodecanoyl substituent at C-6 instead of a decanoyl unit in **7**. The HMBC and COSY data (Figure 1) supported this conclusion. The absolute configuration of **4** was defined by comparison of the optical rotation and NMR data of **4** and **7**, as well as the calculated and experimental ECD spectra (Figure 4). Accordingly, compound **4** (corymbulosin G) was assigned as (2*S*,5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-18,19-di-*O*-acetyl-6-dodecanoyloxy-18,19-epoxy-2-hydroxycyclocleroda-3,13(16),14-triene.

The ¹H and ¹³C NMR spectra of **5** (Tables 1 and 2) were similar to those of compounds **1–4**, which suggested the same tricyclic diterpene skeleton with a different side chain at C-6. However, the lack of a H-2 signal around δ_H 4.40 and the clear deshielding of the C-2 carbon (δ_c 197.8) indicated the presence of a ketone at C-2. This deduction was supported by the presence of an additional C=O absorbance at 1680 cm⁻¹ in the IR spectrum of **5**. Based on the molecular formula, C₃₄H₅₀O₈, from a Na⁺ adduct molecular-related ion at m/z 609.3412 and various NMR data, compound **5** was assigned as a 2-oxo analogue of **1** with a decanoyloxy group at C-6 and the same relative configuration. Compound **7**, for which the absolute configuration was determined as described later, was oxidized by reaction with Dess–Martin periodinate to generate the 2-oxo derivative **5**. All spectroscopic data of the resulting compound were identical with those of the isolated compound **5**. Accordingly, the structure and configuration of **5** (corymbulosin H) were concluded to be (5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-18,19-di-*O*-acetyl-6-dodecanoyloxy-18,19-epoxycyclocleroda-3,13(16),14-trien-2-one.

The structures of known compounds **6** and **7** were identified as corymbulosins B and C, respectively, by comparison of their spectroscopic data with reported values.⁹ Since their

relative configurations were not reported previously, the 2D NOESY spectra of both compounds were evaluated (Supporting Information, Figures S35 and S38). NOESY correlations between H-1/H-6, H-6/H-8, H-2/H-10, H-10/H-12, H-7/H-19, and H-18/H-3' were observed in compound **6**. In compound **7**, similar NOESY correlations were present, but no correlations between H-1/H-6 and H-2/H-10 were found. Application of the modified Mosher ester method revealed that the C-2 stereocenters of **6** and **7** are *R*- and *S*-configured, respectively. Their absolute configurations were confirmed from their ECD spectra (Figure 3), which showed the same Cotton effects as those of the related compounds **1** and **4**, respectively. Thus, stereostructures of **6** and **7** were determined for the first time as (2*R*,5*S*,6*S*,8*R*,9*R*,10*S*,18*R*,19*S*)-18,19-di-*O*-acetyl-6-decanoyloxy-18,19-epoxy-2-hydroxycyclohexa-3,13-(16),14-triene and its 2-epimer, respectively.

The isolated compounds were evaluated for antiproliferative activity against five human tumor cell lines as described in the Experimental Section, including triple-negative breast cancer (MDA-MB-231) cell and the MDR subline KB-VIN. All tested compounds, except **5**, exhibited antiproliferative activity with IC₅₀ values of around 5 μM (Table 3). These results indicated that a hydroxy group at C-2 is more favorable than a ketone for more potent antiproliferative activity, while the size of the ester group at C-6 did not affect the resultant potency.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were determined on a JASCO P-2200 digital polarimeter. ECD spectra were measured on a JASCO J-820 spectrometer. Infrared spectra (IR) were recorded with a Thermo Fisher Scientific NICOLET iS5 FT-TR spectrometer with samples in CH₂Cl₂. NMR spectra were obtained on JEOL JMN-ECA600 and JMN-ECS400 NMR spectrometers with tetramethylsilane as an internal standard, and chemical shifts are stated as δ values. HRMS data were recorded on a JMS-SX102A (FAB) or JMS-T100TD (DART) mass spectrometer. Analytical and preparative TLC were performed on precoated silica gel 60F₂₅₄ and RP-18F₂₅₄ plates (0.25 or 0.50 mm thickness; Merck). MPLC was performed on a Combiflash Rf (Teledyne Isco) instrument with silica gel and C₁₈ cartridges (ODS-25, YMC-DispoPack). Preparative HPLC was conducted on a GL Science recycling system using an InertSustain C₁₈ column (5 μM, 20 × 250 mm).

Plant Material

NCI/NIH (Frederick, MD, USA) provided a crude CH₃OH–CH₂Cl₂ (1:1) extract (N005829) of *L. corymbulosa* bark collected in Peru in the province of Maynas by DC. Daly in February 1988. A voucher specimen of the plant (QT65T0390) was deposited at the Smithsonian Institution (Washington, DC, USA) and reference samples of the extract (N005829) at NCI and Kanazawa University (Kanazawa, Japan). The crude organic extract of (N005829) was evaluated for cytotoxicity by NCI with their in vitro 60-cell tumor screening panel as reported previously.¹¹

Extraction and Isolation

A CH₃OH–CH₂Cl₂ (1:1) extract of the bark of *L. corymbulosa* (12.8 g) was partitioned between H₂O and EtOAc to yield H₂O-soluble (2.2 g) and EtOAc-soluble (7.4 g) fractions. The EtOAc-soluble fraction was subjected to silica gel MPLC (RediSep Rf GOLD High Performance 120 g) with a gradient system [*n*-hexane–EtOAc 90:10 (600 mL) → 80:20 (40 mL) → 75:25 (120 mL) → 65:35 (80 mL) → 60:40 (120 mL) → 55:45 (80 mL) → 45:55 (240 mL) → 30:70 (520 mL) → 10:90 (440 mL) → EtOAc–MeOH 90:10 (120 mL) → MeOH (1400 mL)] to yield 11 fractions, F1–F11. Fraction F7 (3.52 g) was subjected to silica gel column chromatography (CC) eluted with CH₂Cl₂–EtOAc [6:1 (450 mL) → 3:1 (150 mL × 2) → 2:1 (100 mL × 5) → 0:1 (300 mL)], followed by CH₃OH to yield 10 subfractions 7a–j. Subfraction 7e (603.7 mg) was subjected to silica gel CC eluted with *n*-hexane–CH₂Cl₂ (1:1 to 0:1), followed by CH₂Cl₂–EtOAc (2:1) to obtain 10 subfractions, 7e1–10. Subfraction 7e2 (1.8 mg) was purified by HPLC with H₂O–MeOH–CH₃CN–Me₂CO (1:1:1:2) to afford compound **5** (0.7 mg). Subfraction 7e6 (385.9 mg) was purified by MPLC on ODS-25 (YMC-DispoPack AT 12 g) with H₂O–CH₃OH (3:7 to 1:6), followed by recycling preparative HPLC with H₂O–MeOH (1:9) to afford compounds **1** (5.4 mg), **2** (2.4 mg), and **3** (6.0 mg). Subfraction 7e7 (87.5 mg) was purified by MPLC on ODS-25 (YMC-DispoPack AT 12 g) with H₂O–CH₃OH 1:4–1:6, followed by repeated recycling preparative HPLC with H₂O–MeOH (1:9), to provide compounds **3** (0.3 mg), **4** (0.9 mg), **6** (20.7 mg), and **7** (16.7 mg).

Corymbulosin D (1)—Colorless oil; [α]_D²⁵ –6.4 (*c* 0.27, CHCl₃); IR $\nu_{\max}$ (CH₂Cl₂) 3497, 2959, 2929, 2872, 2858, 1761, 1738, 1374, 1225 cm^{–1}; ¹H and ¹³C NMR, Tables 1 and 2; HRFABMS *m/z* 583.3295 [M + Na]⁺ (calcd for C₃₂H₄₈O₈Na, 583.3247).

Corymbulosin E (2)—Colorless oil; [α]_D²⁵ +23.3 (*c* 0.12, CHCl₃); IR $\nu_{\max}$ (CH₂Cl₂) 3458, 2959, 2930, 2876, 2855, 1756, 1715, 1630, 1374, 1228 cm^{–1}; ¹H and ¹³C NMR, Tables 1 and 2; HRFABMS *m/z* 607.3221 [M + Na]⁺ (calcd for C₃₄H₄₈O₈Na, 607.3247).

Corymbulosin F (3)—Colorless oil; [α]_D²⁵ –31.1 (*c* 0.30, CHCl₃); IR $\nu_{\max}$ (CH₂Cl₂) 3458, 2961, 2930, 2874, 2858, 1756, 1716, 1630, 1373, 1227 cm^{–1}; ¹H and ¹³C NMR, Tables 1 and 2; HRFABMS *m/z* 607.3213 [M + Na]⁺ (calcd for C₃₄H₄₈O₈Na, 607.3247).

Corymbulosin G (4)—Colorless oil; [α]_D²⁵ –47.8 (*c* 0.04, CHCl₃); IR $\nu_{\max}$ (CH₂Cl₂) 3452, 2925, 2854, 1757, 1736, 1374, 1225 cm^{–1}; ¹H and ¹³C NMR, Tables 1 and 2; HRFABMS *m/z* 639.3836 [M + Na]⁺ (calcd for C₃₆H₅₆O₈Na, 639.3873).

Corymbulosin H (5)—Colorless oil; [α]_D²⁵ –43.6 (*c* 0.04, CHCl₃); IR $\nu_{\max}$ (CH₂Cl₂) 2956, 2927, 2855, 1761, 1740, 1680, 1374, 1220 cm^{–1}; ¹H and ¹³C NMR, Tables 1 and 2; HRFABMS *m/z* 609.3412 [M + Na]⁺ (calcd for C₃₄H₅₀O₈Na, 609.3403).

General Procedure for Esterification with (S/R)-MTPA-Cl

To a solution of **1** (0.8 mg, 1.4 μ mol) in anhydrous CH₂Cl₂ (0.25 mL) were added Et₃N (1.6 μ L, 10.5 μ mol), DMAP (1.0 mg, 9.2 μ mol), and (*S*)-MTPA-Cl (3.4 μ L, 17.4 μ mol) at rt for 22 h. The mixture was directly purified using preparative TLC with CH₂Cl₂–*n*-hexane (6:1)

to afford the (*R*)-Mosher ester (0.9 mg). The corresponding (*S*)-Mosher ester was obtained by the same procedure using (*R*)-MTPACl.

(*R*)-Mosher Ester of 1—81% yield. ^1H NMR (CDCl_3 , 400 MHz) δ_{H} 7.60–7.36 (5H, m, aromatic protons), 6.55 (1H, t, $J = 1.2$ Hz, H-18), 6.53 (1H, s, H-19), 6.38 (1H, dd, $J = 17.2$, 10.8 Hz, H-14), 6.12 (1H, dd, $J = 4.4$, 1.2 Hz, H-3), 5.69 (1H, m, H-2), 5.09 (1H, d, $J = 17.2$ Hz, H-15), 5.04 (1H, dd, $J = 12.0$, 5.2 Hz, H-6), 5.02 (1H, s, H-16), 4.97 (1H, d, $J = 10.8$ Hz, H-15), 4.90 (1H, s, H-16), 3.61 (3H, s, OMe), 2.53 (1H, dd, $J = 8.8$, 7.6 Hz, H-10), 2.33 (2H, m, H-2'), 2.07 (2H, m, H-12), 2.03 (3H, s, OAc-18), 2.01 (1H, m, H-1), 1.87 (1H, m, H-8), 1.75 (3H, s, OAc-19), 1.27 (8H, m, H-4'-H-7'), 0.93 (3H, d, $J = 6.8$ Hz, H-17), 0.92 (3H, s, H-20), 0.88 (3H, t, $J = 7.2$ Hz, H-8').

(*S*)-Mosher Ester of 1—64% yield. ^1H NMR (CDCl_3 , 400 MHz) δ_{H} 7.59–7.41 (5H, m, aromatic protons), 6.51 (1H, t, $J = 1.2$ Hz, H-18), 6.50 (1H, s, H-19), 6.40 (1H, dd, $J = 18.0$, 10.8 Hz, H-14), 6.11 (1H, m, H-3), 5.66 (1H, m, H-2), 5.08 (1H, d, $J = 18.0$ Hz, H-15), 5.04 (1H, m overlap, H-6), 5.03 (1H, s, H-16), 5.00 (1H, d, $J = 10.8$ Hz, H-15), 4.91 (1H, s, H-16), 3.54 (3H, s, OMe), 2.50 (1H, m, H-10), 2.32 (2H, m, H-2'), 2.07 (2H, m, H-12), 2.02 (3H, s, OAc-18), 2.03 (1H, m, H-1), 1.87 (1H, m, H-8), 1.79 (3H, s, OAc-19), 1.27 (8H, m, H-4'-H-7'), 0.93 (3H, d, $J = 5.6$ Hz, H-17), 0.94 (3H, s, H-20), 0.88 (3H, t, $J = 6.8$ Hz, H-8').

(*R*)-Mosher Ester of 2—100% yield. ^1H NMR (CDCl_3 , 600 MHz) δ_{H} 7.59–7.37 (5H, m, aromatic protons), 7.00 (1H, dd, $J = 12.0$, 11.4 Hz, H-3'), 6.56 (1H, s, H-19), 6.54 (1H, dd, $J = 1.8$, 1.2 Hz, H-18), 6.38 (1H, dd, $J = 17.4$, 10.8 Hz, H-14), 6.11 (1H, dd, $J = 4.8$, 1.8 Hz, H-3), 5.96 (1H, m, H-5'), 5.70 (1H, d, $J = 12.0$ Hz, H-2'), 5.69 (1H, m, H-2), 5.10 (1H, d, $J = 17.4$ Hz, H-15), 5.09 (1H, dd, $J = 12.6$, 4.2 Hz, H-6), 5.02 (1H, s, H-16), 4.97 (1H, d, $J = 10.8$ Hz, H-15), 4.90 (1H, s, H-16), 3.61 (3H, s, OMe), 2.54 (1H, dd, $J = 10.8$, 4.2 Hz, H-10), 2.26 (2H, m, H-6'), 2.08 (1H, m, H-1), 2.02 (3H, s, OAc-18), 2.01 (2H, m, H-12), 1.89 (1H, m, H-8), 1.80 (1H, dt, $J = 12.6$, 4.2, H-7 β), 1.75 (3H, s, OAc-19), 1.41 (2H, m, H-7'), 0.93 (3H, d, $J = 6.6$ Hz, H-17), 0.92 (3H, s, H-20), 0.88 (3H, t, $J = 7.2$ Hz, H-10').

(*S*)-Mosher Ester of 2—44% yield. ^1H NMR (CDCl_3 , 600 MHz) δ_{H} 7.57–7.41 (5H, m, aromatic protons), 6.99 (1H, dd, $J = 12.6$, 11.4 Hz, H-3'), 6.53 (1H, s, H-19), 6.50 (1H, t, $J = 1.2$ Hz, H-18), 6.40 (1H, dd, $J = 17.4$, 10.8 Hz, H-14), 6.10 (1H, m, H-3), 5.95 (1H, m, H-5'), 5.70 (1H, d, $J = 11.4$ Hz, H-2'), 5.66 (1H, m, H-2), 5.08 (1H, d, $J = 17.4$ Hz, H-15), 5.08 (1H, dd, $J = 8.4$, 3.6 Hz, H-6), 5.03 (1H, s, H-16), 4.99 (1H, d, $J = 10.8$ Hz, H-15), 4.91 (1H, s, H-16), 3.54 (3H, s, OMe), 2.51 (1H, dd, $J = 12.0$, 3.0, H-10), 2.25 (2H, m, H-6'), 2.11 (1H, m, H-1), 2.00 (3H, s, OAc-18), 2.03 (2H, m, H-12), 1.89 (1H, m, H-8), 1.79 (3H, s, OAc-19), 1.41 (2H, m, H-7'), 0.94 (3H, s, H-20), 0.93 (3H, d, $J = 7.2$ Hz, H-17), 0.89 (3H, dd, $J = 7.2$, 6.6 Hz, H-10').

(*R*)-Mosher Ester of 3—64% yield. ^1H NMR (CDCl_3 , 400 MHz) δ_{H} 7.57–7.40 (5H, m, aromatic protons), 6.97 (1H, dd, $J = 11.6$, 11.2 Hz, H-3'), 6.51 (1H, s, H-19), 6.46 (1H, s, H-18), 6.44 (1H, dd, $J = 17.2$, 11.2, H-14), 5.95 (1H, m, H-5'), 5.81 (1H, brs, H-3), 5.82 (1H, m, H-2), 5.68 (1H, d, $J = 11.2$ Hz, H-2'), 5.23 (1H, d, $J = 17.2$ Hz, H-15), 5.11 (1H, dd, $J = 11.6$, 4.0 Hz, H-6), 5.06 (1H, d, $J = 11.2$ Hz, H-15), 5.06 (1H, s, H-16), 4.93 (1H, s,

H-16), 3.57 (3H, s, OMe), 2.46 (1H, dd, $J = 13.2, 1.6$ Hz, H-10), 2.25 (2H, m, H-6'), 2.09 (2H, m, H-12), 2.06 (3H, s, OAc-18), 1.90 (3H, s, OAc-19), 1.41 (2H, m, H-7'), 1.00 (3H, s, H-20), 0.92 (3H, d, $J = 6.4$ Hz, H-17), 0.88 (3H, t, $J = 6.4$ Hz, H-10').

(S)-Mosher Ester of 3—65% yield. ^1H NMR (CDCl_3 , 400 MHz) δ_{H} 7.57–7.41 (5H, m, aromatic protons), 6.98 (1H, dd, $J = 12.0, 11.6$ Hz, H-3'), 6.51 (1H, s, H-19), 6.48 (1H, t, $J = 1.2$ Hz, H-18), 6.44 (1H, dd, $J = 18.0, 10.8$, H-14), 5.95 (1H, m, H-5'), 5.94 (1H, brs, H-3), 5.82 (1H, m, H-2), 5.69 (1H, d, $J = 11.6$ Hz, H-2'), 5.22 (1H, d, $J = 18.0$ Hz, H-15), 5.12 (1H, dd, $J = 12.0, 4.0$ Hz, H-6), 5.06 (1H, d, $J = 10.8$ Hz, H-15), 5.06 (1H, s, H-16), 4.92 (1H, s, H-16), 3.57 (3H, s, OMe), 2.44 (1H, dd, $J = 13.6, 2.0$ Hz, H-10), 2.26 (2H, m, H-6'), 2.08 (2H, m, H-12), 2.07 (3H, s, OAc-18), 1.90 (3H, s, OAc-19), 1.41 (2H, m, H-7'), 0.96 (3H, s, H-20), 0.91 (3H, d, $J = 6.4$ Hz, H-17), 0.89 (3H, t, $J = 6.8$ Hz, H-10').

(R)-Mosher Ester of 6—66% yield. ^1H NMR (CDCl_3 , 400 MHz) δ_{H} 7.60–7.35 (5H, m, aromatic protons), 6.56 (1H, t, $J = 1.2$ Hz, H-18), 6.53 (1H, s, H-19), 6.38 (1H, dd, $J = 17.2, 10.8$ Hz, H-14), 6.12 (1H, dd, $J = 4.4, 1.2$ Hz, H-3), 5.69 (1H, m, H-2), 5.09 (1H, d, $J = 17.2$ Hz, H-15), 5.04 (1H, dd, $J = 11.6, 4.8$ Hz, H-6), 5.02 (1H, s, H-16), 4.97 (1H, d, $J = 10.8$ Hz, H-15), 4.90 (1H, s, H-16), 3.61 (3H, s, OMe), 2.53 (1H, dd, $J = 8.4, 8.0$ Hz, H-10), 2.33 (2H, m, H-6'), 2.07 (2H, m, H-12), 2.03 (3H, s, OAc-18), 1.85 (1H, m, H-8), 1.75 (3H, s, OAc-19), 1.50 (1H, m, H-11), 0.93 (3H, d, $J = 6.8$ Hz, H-17), 0.92 (3H, s, H-20), 0.88 (3H, dd, $J = 7.2, 6.8$ Hz, H-10').

(S)-Mosher Ester of 6—65% yield. ^1H NMR (CDCl_3 , 400 MHz) δ_{H} 7.58–7.40 (5H, m, aromatic protons), 6.51 (1H, t, $J = 1.2$ Hz, H-18), 6.50 (1H, s, H-19), 6.40 (1H, dd, $J = 17.6, 10.8$ Hz, H-14), 6.10 (1H, dd, $J = 4.0, 1.2$ Hz, H-3), 5.66 (1H, m, H-2), 5.07 (1H, d, $J = 17.6$ Hz, H-15), 5.02 (1H, m overlap, H-6), 5.03 (1H, s, H-16), 4.99 (1H, d, $J = 10.8$ Hz, H-15), 4.91 (1H, s, H-16), 3.54 (3H, s, OMe), 2.50 (1H, dd, $J = 10.4, 6.8$ Hz, H-10), 2.32 (2H, m, H-6'), 2.09 (2H, m, H-12), 2.02 (3H, s, OAc-18), 1.85 (1H, m, H-8), 1.79 (3H, s, OAc-19), 1.52 (1H, m, H-11), 0.94 (3H, s, H-20), 0.93 (3H, d, $J = 6.0$ Hz, H-17), 0.88 (3H, dd, $J = 7.2, 6.4$ Hz, H-10').

(R)-Mosher Ester of 7—23% yield. ^1H NMR (CDCl_3 , 600 MHz) δ_{H} 7.56–7.41 (5H, m, aromatic protons), 6.48 (1H, s, H-19), 6.46 (1H, m, H-18), 6.43 (1H, dd, $J = 17.4, 10.8$ Hz, H-14), 5.81 (1H, brs, H-3), 5.80 (1H, m, H-2), 5.22 (1H, 2, $J = 17.4$ Hz, H-15), 5.08 (1H, dd, $J = 12.6, 4.8$, H-6), 5.060 (1H, d, $J = 10.8$ Hz, H-15), 5.060 (1H, s, H-16), 4.92 (1H, s, H-16), 3.57 (3H, s, OMe), 2.44 (1H, dd, $J = 14.4, 3.0$ Hz, H-10), 2.30 (2H, m, H-2'), 2.06 (3H, s, OAc-18), 1.90 (3H, s, OAc-19), 1.79 (1H, dt, $J = 14.4, 4.8$ Hz, H-7 β), 0.99 (3H, s, H-20), 0.92 (3H, d, $J = 7.2$ Hz, H-17), 0.87 (3H, t, $J = 7.2$ Hz, H-10').

(S)-Mosher Ester of 7—87% yield. ^1H NMR (CDCl_3 , 600 MHz) δ_{H} 7.56–7.43 (5H, m, aromatic protons), 6.481 (1H, t, $J = 1.8$ Hz, H-18), 6.477 (1H, s, H-19), 6.43 (1H, dd, $J = 18.0, 10.8$ Hz, H-14), 5.95 (1H, brs, H-3), 5.81 (1H, m, H-2), 5.22 (1H, 2, $J = 18.0$ Hz, H-15), 5.09 (1H, dd, $J = 12.0, 4.2$, H-6), 5.057 (1H, d, $J = 10.8$ Hz, H-15), 5.055 (1H, s, H-16), 4.92 (1H, s, H-16), 3.57 (3H, s, OMe), 2.43 (1H, dd, $J = 13.8, 3.0$ Hz, H-10), 2.31 (2H, m, H-2'), 2.17 (1H, m, H-1), 2.08 (3H, s, OAc-18), 1.90 (3H, s, OAc-19), 1.87 (1H, m,

H-8), 1.75 (1H, dt, $J = 13.2, 4.2$ Hz, H-7 β), 1.50 (1H, m, H-11), 0.95 (3H, s, H-20), 0.90 (3H, d, $J = 6.6$ Hz, H-17), 0.88 (3H, dd, $J = 7.2, 6.6$ Hz, H-10').

Oxidation of Corymbulosins C (7)

To a solution of **7** (3.3 mg) in anhydrous CH₂Cl₂ (0.50 mL) were added pyridine (1.8 μ L, 22.4 μ mol) and Dess–Martin periodinate (4.7 mg, 11.2 μ mol) at 0 °C. The mixture was stirred at rt for 4 h. The whole was filtered through Celite, and washed with Et₂O. The filtrate was concentrated in vacuo, and purified using preparative TLC with EtOAc–CH₂Cl₂ (1:9) to provide oxidized compound **5** (2.1 mg, 64%). All physical and spectroscopic data of the obtained compound were identical with those of the isolated compound **5**.

Calculated ECD Spectra

The most stable conformer of **1** was calculated using Spartan'14 by a preliminary conformational analysis with the MMFF94 force field followed by geometry optimization using Gaussian09¹² with the density functional theory (DFT) B3LYP/6-31G(d). The ECD spectrum in acetonitrile was calculated by the time-dependent DFT (TDDFT) with the CAM-B3LYP/SVP. The solvent effect was introduced through the polarizable continuum model (PCM). Ten low-lying excited states were calculated. The calculated spectrum was shown using GaussView 5.0.920 with the peak half-width at half height being 0.333 eV. The calculated spectrum was shifted by +10 nm to match the experimental spectrum.

Assay for Antiproliferative Activity

The antiproliferative activity of the compounds was determined by the sulforhodamine B (SRB) assay, as described previously.¹¹ Briefly, cell suspensions were seeded on 96-well microtiter plates at a density of 4,000–12,000 cells per well and treated with the test compounds. After a 72-h culture with the compounds, the cells were fixed in 10% trichloroacetic acid and then stained with 0.04% SRB. The absorbance at 515 nm of 10 mM Tris base solubilized with protein-bound dye was measured using a microplate reader (ELx800, BioTek) operated by Gen5 software (BioTek). Then, IC₅₀ data were calculated statistically (MS Excel) from at least three independent experiments performed with duplication. The following human tumor cell lines were used in this study: A549 (lung carcinoma), MDA-MB-231 (triple-negative breast cancer), MCF-7 (estrogen receptor-positive and HER2-negative breast cancer), KB (originally isolated from epidermoid carcinoma of the nasopharynx), and KB-VIN (vincristine-resistant KB subline showing MDR phenotype by overexpressing P-gp). The first four cell lines mentioned above were obtained from the Lineberger Comprehensive Cancer Center (UNC-CH) or from ATCC (Manassas, VA). The last cell line, KB-VIN, was a generous gift from Y.-C. Cheng of Yale University. The cells were cultured in RPMI-1640 medium supplemented with 2 mM L-glutamine and 25 mM HEPES (Corning), containing 10% fetal bovine serum (Corning), 100 μ g/mL streptomycin, and 100 IU penicillin (Corning). KB-VIN stock cells were maintained in the presence of 100 nM vincristine. Paclitaxel was used as an experimental control.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HMBC/COSY
(400 MHz)

NOESY
(400 MHz)

Figure 1.
Selected HMBC correlations (arrows), COSY connectivities (bold lines), and key NOESY correlations for 1–5.

Figure 2.
 δ_H ($S-R$) values (ppm) calculated from the 2- O -(S)- and 2- O -(R)-Mosher esters of compounds **1**–**3**, **6**, and **7**.

Figure 3.
Experimental ECD spectra of compounds 1–7 in acetonitrile.

Figure 4.
Experimental and calculated ECD spectra of compound **4** in acetonitrile.

Corymbulosin D (**1**) R = octanoylCorymbulosin E (**2**) R = (2Z,4Z)-deca-2,4-dienoylCorymbulosin B (**6**) R = decanoyl Corymbulosin F (**3**) R = (2Z,4Z)-deca-2,4-dienoylCorymbulosin G (**4**) R = dodecanoylCorymbulosin C (**7**) R = decanoyl Corymbulosin H (**5**)

Chart 1.

Table 1

¹H NMR Spectroscopic Data of Compounds 1–5

position	1 ^a (CDCl ₃) δ _H (J in Hz)	2 ^a (CD ₃ OD) δ _H (J in Hz)	3 ^b (CD ₃ OD) δ _H (J in Hz)	4 ^a (CDCl ₃) δ _H (J in Hz)	5 ^a (CDCl ₃) δ _H (J in Hz)
1	2.01 m	2.10 m	2.19 m	2.24 ddd (13.2, 6.6, 3.0)	2.55 dd (17.4, 5.4)
2	1.69 m	1.95 m	1.76 m	1.70 m	2.61 dd (17.4, 13.8)
3	4.44 m	4.39 m	4.39 m	4.49 m	
6	6.00 brd (4.2)	6.02 brd (3.6)	5.94 brs	5.94 m	6.14 brs
6	4.98 dd (11.4, 4.8)	5.12 dd (13.2, 4.2)	5.28 dd (11.6, 4.8) ^c	5.17 dd (12.0, 4.2)	5.13 dd (13.2, 3.6)
7	1.68 m	1.76 q (13.2)	1.74 m	1.67 m	1.72 q (13.2)
		1.68 dt (13.2, 4.2)		1.75 dt (13.2, 4.2)	1.82 dt (13.2, 3.6)
8	1.89 m	1.95 m	2.01 m	1.95 m	1.99 m
10	2.34 dd (13.2, 3.6)	2.47 dd (13.8, 3.0)	2.39 dd (13.6, 2.8)	2.32 m	2.86 dd (13.8, 5.4)
11	1.52 m	1.53 dt (13.2, 4.2)	1.51 m	1.50 m	1.54 m
	1.26 m ^c	1.32 m	1.31 m	1.20 m	1.29 m ^e
12	2.11 m	2.16 m	2.13 m	2.07 m	2.09 m
14	6.43 dd (17.4, 10.8)	6.46 dd (17.4, 10.8)	6.46 dd (17.6, 11.2)	6.43 dd (17.4, 10.8)	6.43 dd (18.0, 10.8)
15	5.22 d (17.4)	5.29 d (17.4)	5.27 d (17.6)	5.21 d (17.4)	5.21 d (18.0)
	5.04 d (10.8)	5.04 d (10.8)	5.04 d (11.2)	5.04 d (10.8)	5.05 d (10.8)
16	5.05 s	5.01 s	5.01 s	5.05 s	5.07 s
	4.94 s	4.95 s	4.94 s	4.93 s	4.93 s
17	0.92 d (6.6)	0.96 d (6.6)	0.96 d (6.8)	0.92 d (6.6)	0.96 d (6.6)
18	6.51 t (1.8)	6.39 dd (1.8, 1.2)	6.35 dd (2.0, 1.2)	6.46 dd (2.4, 1.2)	6.65 m
19	6.54 s	6.52 s	6.45 s	6.48 s	6.62 s
20	0.99 s	1.02 s	1.03 s	0.98 s	0.95 s
2'	2.31 m	5.70 d (11.4)	5.71 d (11.6)	2.30 m	2.31 m
3'	1.61 m	7.08 dd (12.0, 11.4)	7.08 dd (12.4, 11.6)	1.60 m	1.61 m
4'	1.28 m ^c	7.26 dd (12.0, 10.8)	7.28 dd (12.4, 12.0)	1.27 m ^d	1.27 m ^e
5'	1.28 m ^c	5.94 m	5.93 m	1.27 m ^d	1.27 m ^e
6'	1.28 m ^c	2.30 m	2.30 m	1.27 m ^d	1.27 m ^e

position	1 ^a δ_{H} (CDCl ₃) (<i>J</i> in Hz)	2 ^a δ_{H} (CD ₃ OD) (<i>J</i> in Hz)	3 ^b δ_{H} (CD ₃ OD) (<i>J</i> in Hz)	4 ^a δ_{H} (CDCl ₃) (<i>J</i> in Hz)	5 ^a δ_{H} (CDCl ₃) (<i>J</i> in Hz)
7'	1.28 m ^c	1.43 m	1.43 m	1.27 m ^d	1.27 m ^e
8'	0.87 t (7.2)	1.32 m	1.31 m	1.27 m ^d	1.27 m ^e
6'				1.27 m ^d	1.27 m ^e
10'		0.91 dd (7.2, 6.6)	0.91 t (6.8)	1.27 m ^d	0.88 t (7.2)
11'				1.27 m ^d	
12'				0.88 t (7.2)	
OAc-18	2.07 s	2.03 s	2.04 s	2.06 s	2.07 s
OAc-19	1.90 s	1.86 s	1.85 s	1.90 s	1.91 s

^a 600 MHz.

^b 400 MHz.

^{c-e} Overlapping signals.

Table 2

¹³C NMR Spectroscopic Data (100 MHz) of Compounds 1–5

	1 (CDCl ₃)	2 (CD ₃ OD)	3 (CD ₃ OD)	4 (CDCl ₃)	5 (CDCl ₃)
position	δ _C	δ _C	δ _C	δ _C	δ _C
1	29.5	30.5	31.2	30.7	35.5
2	63.8	64.3	69.2	68.6	197.8
3	126.4	128.4	131.0	129.2	125.6
4	142.6	142.5	141.8	141.7	163.0
5	52.2	53.8	53.8	52.3	53.4
6	73.7	74.9	76.2	74.9	73.8
7	33.1	34.4	34.5	33.4	33.0
8	36.7	38.1	38.2	36.9	36.3
9	37.4	38.6	39.1	38.0	37.9
10	36.2	37.6	43.3	42.1	41.9
11	27.9	29.4	28.9	27.5	27.2
12	23.8	25.1	25.0	23.7	23.7
13	145.2	147.2	147.0	145.0	144.8
14	140.3	141.7	141.58	140.3	140.1
15	112.6	113.2	113.2	112.5	112.7
16	115.4	115.6	115.7	115.4	115.5
17	15.6	16.0	15.9	15.6	15.5
18	95.4	96.7	96.0	94.7	94.4
19	98.3	99.7	99.1	97.7	98.1
20	25.5	26.0	25.9	25.6	24.7
1'	173.3	167.0	166.9	173.4	172.9
2'	34.7	117.5	117.5	34.8	34.6
3'	24.8	141.7	141.64	24.8	24.6
4'	29.1	125.6	125.6	29.16 ^a	29.1 ^b
5'	28.9	143.5	143.5	29.22 ^a	29.17 ^b
6'	31.6	28.4	28.4	29.4 ^a	29.22 ^b
7'	22.6	30.3	30.2	29.5 ^a	29.4 ^b

position	1 (CDCl ₃) δ_C	2 (CD ₃ OD) δ_C	3 (CD ₃ OD) δ_C	4 (CDCl ₃) δ_C	5 (CDCl ₃) δ_C
8'	14.1	32.6	32.5	29.61 ^a	31.8
9'		23.6	23.6	29.61 ^a	22.7
10'		14.4	14.4	31.9	15.5
11'				22.7	
12'				14.1	
OAc-18	170.1	172.0	171.9	169.9	169.5
Me	21.3	21.1	21.0	21.2	21.0
OAc-19	170.0	171.7	171.5	169.9	169.5
Me	21.7	21.8	21.8	21.6	21.5

^{a,b} Assignments may be interchanged.

Table 3

Antiproliferative Activity of Diterpenoids 1–3 and 5–7

compound	Cell line ^a (IC ₅₀ μ M) ^b				
	A549	MDA-MB-231	MCF-7	KB	KB-VIN
1	4.7	5.1	4.9	5.0	4.9
2	4.6	4.8	4.8	4.8	4.9
3	4.8	5.1	5.2	4.9	4.8
5	>10	>10	8.3	5.9	9.8
6	4.7	5.2	5.0	5.0	4.9
7	5.3	5.4	5.4	4.5	4.8
paclitaxel (nM)	6.2	8.8	10.4	6.3	1926.0

^a A549 (lung carcinoma), MDA-MB-231 (triple-negative breast cancer), MCF-7 (estrogen receptor-positive and HER2-negative breast cancer), KB (originally isolated from epidermoid carcinoma of the nasopharynx), KB-VIN (P-gp-overexpressing MDR subline of KB).

^b Antiproliferative activity expressed as IC₅₀ values for each cell line, the concentration of compound that caused 50% reduction relative to untreated cells determined by the SRB assay.