New Diterpenes from the Caribbean Sponge *Epipolasis reiswigi*

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Received May 8, 2001

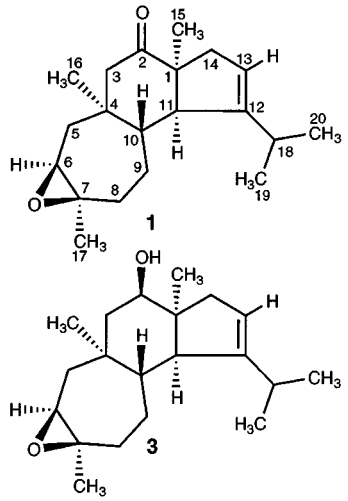
Two diterpenes, epipolone (**1**) and epipolol (**3**), produced by terpenoid pathways leading to a tricarbocyclic structure with an irregular "head to tail" isoprene configuration, have been isolated from the Caribbean marine sponge *Epipolasis reiswigi* collected in Puerto Rico. The structures of **1** and **3** were elucidated largely by 1D and 2D NMR methods and chemical conversion.

Introduction

Not surprisingly, marine sponges were among the earliest marine invertebrates to attract the attention of natural product chemists. These animals are renowned among marine organisms for their ability to produce novel natural products with unique structures and biological activities.[2] Representative compounds include polyketides, cyclic peptides, alkaloids, pigments, and a vast array of terpene metabolites.[3] During our investigation of bioactive products from marine organisms from the Caribbean Sea, we have isolated two diterpenes from the Puerto Rican sponge *Epipolasis reiswigi* Topsent (class Demospongiae, order Halichondria) collected by scuba at 40 m. Previous work by Kashman and coworkers with *E. reiswigi* from Venezuela revealed the presence of perhydroazulene diterpenes showing potent in vitro antiviral activity.[4] Here, we report the isolation, structure elucidation, and results of three primary screening bioassays of these compounds.

Results and Discussion

After filtration, the $CHCl_3$−MeOH (1:1) extract of freeze-dried *E. reiswigi* (90 g) was partitioned between hexane and water, and the organic layer was fractionated on silica gel with a hexane/EtOAc system. The fractions containing terpenoid metabolites were separately purified by repetitive normal-phase chromatography and flash chromatography on ODS to give epipolone (**1**, 6.6 mg, 4.0 × 10^{-2} % dry wt) and epipolol (**3**, 8.5 mg, 5.2 × 10^{-2} % dry wt), and the known terpenes reiswigin B (**4**, 555 mg; 3.4% dry wt), (+)-curcuphenol (**5**, 173 mg, 1.1% dry wt), and (+)-curcudiol (**6**, 446 mg, 2.7% dry wt).[4,5] The structures of these metabolites were determined by interpretation of the 1D and 2D NMR (^{13}C, ^{1}H, $^{1}H-^{1}H$ COSY, HMQC, HMBC, and NOESY) and IR, UV, and accurate mass measurements (HREI-MS).



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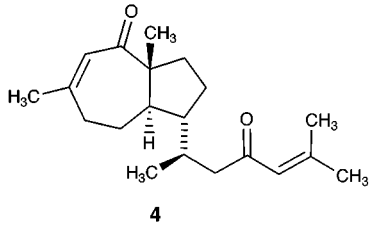

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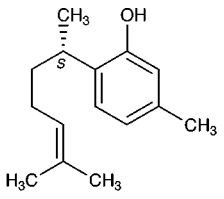

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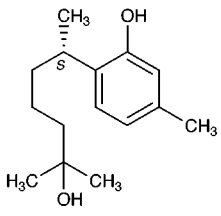

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Epipolone (**1**), a colorless oil, analyzed for $C_{20}H_{30}O_2$ on the basis of its combined HREIMS ([M^+] *m/z* 302.2243, Δ 0.3 mmu) and ^{13}C NMR spectral features (Table 1), which required six degrees of unsaturation. The IR spectrum of **1** showed intense absorptions for ketone (1696 cm^{-1}), olefin (1633 cm^{-1}), and epoxy (1258 cm^{-1}) functionalities. The C=C and C=O double bonds were

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(1) Graduate student sponsored by the NIH-MBRS Program of the University of Puerto Rico.

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(3) Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1−49 and previous reports in this series.

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10.1021/jo0104734 CCC: $20.00 © 2001 American Chemical Society
Published on Web 08/29/2001

Table 1. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of Epipolone (1) in $CDCl_3$[a]

position	δ_H, mult, intgr (J, Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1		54.5 (s)			H3α*β*, H11, H13, H14α*β*, Me-15
2		214.8 (s)			H3α*β*, H14*β*, Me-15
3α	2.02, d, 1H (14.4)	54.0 (t)	H3*β*	H3*β*	Me-16
3*β*	2.50, d, 1H (14.4)		H3α, Me-16	H3α, H5*β*, H10	
4		39.6 (s)			H3α*β*, H5α*β*, H10, Me-16
5α	1.91, m, 1H	43.5 (t)	H5*β*, H6	H5*β*, H6	H3α*β*, H6, Me-16
5*β*	1.44, m, 1H		H5α, H6, Me-16	H3*β*, H5α	
6	2.73, br t, 1H (7.5)	59.6 (d)	H5α*β*	H5α, Me-16, Me-17	H5α*β*, H8α*β*, Me-17
7		60.2 (s)			H5α*β*, H8α*β*, Me-17
8α	2.00, m, 1H	34.6 (t)	H8*β*, H9α*β*	H8*β*, Me-17	H9α*β*, H10, Me-17
8*β*	1.46, m, 1H		H8α, H9α*β*	H8α	
9α	1. 97, m, 1H	26.0 (t)	H8α*β*, H9*β*, H10	H9*β*, H18	H8α*β*, H10, H11
9*β*	1.41, m, 1H		H8α*β*, H9α, H10	H9α	
10	1.48, m, 1H	55.0 (d)	H9α*β*, H11, Me-16	H3*β*	H3α*β*, H5α*β*, H8α*β*, H9α*β*, H11, Me−16
11	2.17, br d, 1H (9.6)	59.2 (d)	H10	Me-15, Me-16, Me-20	H9α*β*, H10, H13, Me-15, H18
12		156.2 (s)			H11, H13, H14α*β*, H18, Me-19, Me-20
13	5.38, br s, 1H	120.5 (d)	H14α*β*	Me-19	H11, H14α*β*, H18
14α	1.87, dd, 1H (2.4, 13.5)	42.6 (t)	H13, H14*β*	H14*β*	H11, H13, Me-15
14*β*	2.58, br d, 1H (13.5)		H13, H14α	H14α	
15	1.08, s, 3H	22.5 (q)		H11, Me-16	H11, H14α*β*
16	0.87, br s, 3H	16.9 (q)	H3*β*, H5*β*, H10	H6, H11, Me-15	H3α*β*, H5α*β*, H10
17	1.33, s, 3H	22.1 (q)		H6, H8α	H6, H8α*β*
18	2.47, m, 1H	30.3 (d)	Me-19, Me-20	H9α, Me-19, Me-20	H13, Me-19, Me-20
19	1.13, d, 3H (6.6)	21.6 (q)	H18	H13, H18	Me-20
20	0.99, d, 3H (6.9)	22.4 (q)	H18	H11, H18	Me-19

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. [b] ^{13}C NMR multiplicities were obtained from an Attached Proton Test (APT) experiment. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6, 8 Hz.

not conjugated since the UV showed only end absorption. The ^{1}H NMR spectrum in $CDCl_3$ showed a low field signal at δ 5.38 (br s, 1H) suggesting the presence in **1** of a trisubstituted olefin functionality. Other relevant features in the ^{1}H NMR spectrum were: a signal for an epoxymethine [δ 2.73 (br t, 1H, J = 7.5 Hz), a characteristic AB pattern composed of two doublets at δ 2.50 and 2.02 (each 1H, J = 14.4 Hz) suggesting the presence of a pair of isolated methylene protons flanked by a ketone and a quaternary carbon, and five methyl groups: two secondary [δ 1.13 (d, 3H, J = 6.6 Hz); 0.99 (d, 3H, J = 6.9 Hz) and three tertiary [δ 1.33 (s, 3H); 1.08 (s, 3H); 0.87 (br s, 3H)]. The ^{13}C NMR spectrum exhibited 20 signals (five CH_3, five CH_2, five CH, and five C) whose chemical shift values and multiplicities suggested the presence of a monosubstituted cyclopentene [δ 156.2 (s) and 120.5 (d)], a cyclic ketone [δ 214.8 (s)], and a trisubstituted epoxide [δ 60.2 (s) and 59.6 (d)]. The presence of these features requires **1** to contain three carbocyclic rings. Analysis of the NMR data allowed the structure of epipolone to be constructed. Overall, proton COSY correlations revealed five coupling sequences, one involving the isolated methylene protons at C-3 and another the methylene and oxymethine protons at C-5 and C-6, respectively. Additional spin systems encompassed the aliphatic protons at C-8, C-9, C-10, and C-11; one involving the olefin and methylene protons, respectively, at C-13 and C-14; and the last involving the protons of an isopropyl group at C-18, C-19, and C-20. Another series of COSY correlations allowed the protons at C-16 to be correlated along the A/B ring junction

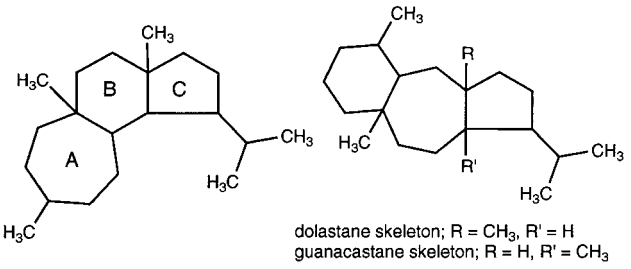

Figure 1. Side-by-side comparison of the tricarbocyclic skeleton of compounds **1**−**3** and the dolastane and guanacastane classes of diterpenes.

(Figure 1) through H-3*β*, H-5*β*, and to the proton at the angular C-10 position.

Heterocorrelation NMR methods, specifically HMQC and HMBC measurements, allowed rings A, B, and C to be fully constructed (Figure 1). The presence of tertiary methyl groups at C-1, C-4, and C-7 assisted in identifying these linkages by HMBC methods. HMBC correlations between H-6 and C-5 and C-17; between H_2-5α*β* and C-4, C-6, C-10, and C-16; between H_2-8α*β* and C-6, C-7, C-10, and C-17; between H-10, and C-4, C-8, and C-16; and between H_3-17 and C-6, C-7, and C-8, clearly established the presence of the 1,4-dimethyl-1,2-epoxycycloheptane structure for epipolone. Additional HMBC assignments were possible from C-18 to C-20, thus completing the terminal end of the molecule.

In general, the region involving rings B and C was easier to analyze because the ^{1}H NMR signals were sharper and well dispersed in $CDCl_3$. The isolated methylene signals at δ 2.50 and 2.02 (H_2-3) showed HMBC correlations to C-1, C-2, C-4, C-5, C-10, and C-16, which placed C-3 next to the A/B ring junction. Correlations from both H_2-3 signals and H_3-15 to the carbonyl signal at δ 214.8 required the carbonyl group to be positioned

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Table 2. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of Isoepipolone (2) in $CDCl_3$[a]

position	δ_H, mult, intgr (J, Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1		54.4 (s)			H3α, H11, H13, Me-15
2		215.0 (s)			H3α*β*, Me-15
3α	2.05, br d, 1H (14.0)	54.5 (t)	H3*β*		H5α, Me-16
3*β*	2.53, br d, 1H (14.0)		H3α, Me-16	H5*β*, H10	
4		38.8 (s)			H3α*β*, H5α, Me-16
5α	1.78, dd, 1H (4.9, 13.8)	49.7 (t)	H5*β*, H6	Me-16	H3α*β*, Me-16
5*β*	1.63, dd, 1H (10.7, 13.8)		H5α, H6	H3*β*	
6	4.29, dd, 1H (4.9, 10.7)	70.8 (d)	H5α*β*	Me-16, H17α	H5α*β*, H17α*β*
7		153.6 (s)			H5α
8α	2.36, m, 1H	31.8 (t)	H8*β*, H9α*β*	H17*β*	H17α*β*
8*β*	2.34, m, 1H		H8α, H9α*β*		
9α	2.06, m, 1H	30.9 (t)	H8α*β*, H9*β*, H10	H18, Me-20	H11
9*β*	1.41, m, 1H		H8α*β*, H9α, H10		
10	1.49, m, 1H	52.0 (d)	H9α*β*, H11	H3*β*	H3α*β*, H5α, H9α, H11, Me−16
11	2.15, br d, 1H (9.3)	59.6 (d)	H10	Me-15, Me-16	H13, Me-15
12		156.1 (s)			H11, Me-19, Me-20
13	5.37, m, 1H	119.9 (d)	H14α*β*	Me-19	H11
14α	1.90, dd, 1H (2.9, 16.4)	42.7 (t)	H13, H14*β*	Me-15	H11, Me-15
14*β*	2.58, br d, 1H (16.4)		H13, H14α		
15	1.08, s, 3H	22.8 (q)		H11, H14α	H11
16	0.80, br s, 3H	17.4 (q)	H3*β*	H5α, H6, H11	H3*β*, H5α
17α	5.06, br s, 1H	112.4 (t)	H17*β*	H6, H17*β*	
17*β*	4.95, br s, 1H		H17α	H8α, H17α	
18	2.43, m, 1H	29.7 (d)	Me-19, Me-20	H9α, Me-19, Me-20	Me-19, Me-20
19	1.15, d, 3H (6.5)	21.6 (q)	H18	H13, H18	Me-20
20	1.00, d, 3H (6.8)	22.2 (q)	H18	H9α, H18	Me-19

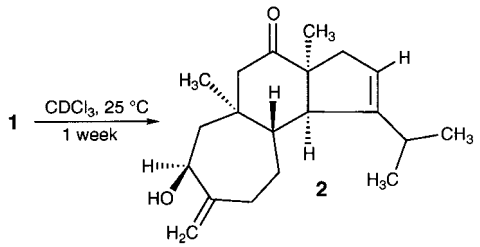
[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. [b] ^{13}C NMR multiplicities were obtained from an Attached Proton Test (APT) experiment. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6, 8 Hz.

at C-2, and correlations from H_3-15 to C-1, C-11, and C-14 placed the methyl group at the C-1 angular position. The angular H-11 methine signal at δ 2.17 (br d, 1H, J = 9.6 Hz) showed correlations to C-1, C-9, C-10, and C-14, and to the vinyl carbon signals at δ 156.2 (s, C-12) and 120.5 (d, C-13): these data established the presence of a six-membered ring and allowed the complete A/B ring junction to be elucidated. The correlations from both H_2-9 signals at δ 1.97 and 1.41 (each 1H, m) to C-8, C-10, and C-11 confirmed this contention. The HMBC correlations from the vinyl proton in **1** at δ 5.38 (H-13) to C-1, C-11, C-14, and to its partner's carbon signal C-12 allowed the cyclopentene structure and thus the B/C ring junction to be fully constructed. Correlations from H-18 at δ 2.47 (m, 1H) to the olefinic carbons C-12 and C-13 required the presence of an isopropyl group, the position of which was defined by the correlations from H_3-19 and H_3-20 to C-12 and from H-18 to C-11. Thus, the planar structure of **1** was determined.

The relative stereochemistry of epipolone (**1**) was determined using a combination of NMR methods (COSY, NOESY, ^{1}H−^{1}H NMR coupling constants) coupled with a molecular modeling study.[6] The H-11 signal showed a proton COSY correlation (J = 9.6 Hz) only to the H-10 signal at δ 1.48. If we arbitrarily assume that H-11 is in a pseudoaxial conformation below the plane of the molecule, we can deduce that H-10 must be above the plane in a trans pseudodiaxial conformation to H-11. Strong NOESY correlations between H-11, H_3-15, and H_3-16 established the spatial proximities to these protons on the bottom face of the molecule. Similarly, NOESY interactions between H-6, H_3-16, and H_3-17 allowed the assignment of these protons to the bottom face of **1**.

(6) For energy calculations the DISCOVER module of the Insight II package (Molecular Simulation Inc.) running on a Silicon Graphics O_2 workstation was operated.

Scheme 1



Further analysis of the proton COSY spectrum allowed us to trace the long range ^{1}H−^{1}H couplings of H-10 to the H_3-16 methyl group. No correlation, however, was observed between the latter protons upon interpretation of the NOESY data, implying that both lie on opposite sides of the A/B ring junction. On the other hand, a pronounced NOESY correlation between the H-10 and H-3*β* signals indicates that H-10 is also on the upper face of the ring, resulting in the geometry proposed. Data were not obtained to define the absolute stereochemistry of epipolone (**1**).

When allowed to stand in $CDCl_3$ solution, epipolone (**1**) isomerized to give a product named isoepipolone (**2**, Scheme 1) whose structure was assigned by combined spectroscopic methods, including extensive 2D NMR experiments. Applying these combined NMR methods resulted in the unambiguous assignment of all protons and carbons as listed in Table 2, and allowed the complete structure for **2** to be assigned. Isoepipolone was obtained as a colorless oil, and the molecular formula $C_{20}H_{30}O_2$ obtained from HREIMS indicated that **2** was an isomer of epipolone (**1**). Moreover, comparison of the NMR spectra of **2** with those of **1** confirmed the overall similarity between their structures (see Tables 1 and 2). An allylic hydroxymethine at δ 4.29 (dd, 1H, J = 4.9, 10.7 Hz) rather than an epoxymethine at C-6 was deduced from the ^{1}H NMR spectrum, which also showed two broad

Table 3. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectral Data of Epipolol (3) in $CDCl_3$[a]

position	δ_H, mult, intgr (*J*, Hz)	δ_C (mult)[b]	^{1}H−^{1}H COSY	NOESY	HMBC[c]
1		48.7 (s)			H3α, H11, H13, Me-15
2	3.80, dd, 1H (5.3, 12.0)	72.6 (d)	H3α*β*	H3α, Me-15, Me-16	H3α*β*, Me-15
3α	1.52, dd, 1H (5.3, 12.6)	46.7 (t)	H2, H3*β*	H2	H10, Me-16
3*β*	1.39, br dd, 1H (12.0, 12.6)		H2, H3α		
4		37.9 (s)			H3α*β*, H5α, H10, Me-16
5α	1.97, dd, 1H (2.6, 14.0)	44.2 (t)	H5*β*, H6	H6	H3α*β*, H6, Me-16
5*β*	1.30, m, 1H		H5α, H6		
6	2.74, br t, 1H (7.2)	60.4 (d)	H5α*β*	H5α, Me-16, Me-17	H5α*β*, H8α, Me-17
7		60.2 (s)			H5α*β*, H8α, Me-17
8α	1.95, dd, 1H (3.6, 14.3)	35.2 (t)	H8*β*, H9α*β*	Me-17	H10, Me-17
8*β*	1.41, m, 1H		H8α, H9α*β*		
9α	1.78, m, 1H	25.3 (t)	H8α*β*, H9*β*, H10	H18	H11
9*β*	1.27, m, 1H		H8α*β*, H9α, H10		
10	1.05, m, 1H	55.3 (d)	H9α*β*, H11		H3α*β*, H5α, H8α, H9α*β*, H11, Me−16
11	1.83, br d, 1H (10.6)	56.1 (d)	H10	Me-15, Me-16, Me-20	H10, H13, Me-15
12		157.2 (s)			H11, H14α*β*, Me-19, Me-20
13	5.36, br s, 1H	120.7 (d)	H14α*β*	Me-19	H11, H14*β*, H18
14α	1.66, dd, 1H (3.5, 15.5)	35.6 (t)	H13, H14*β*	Me-15	H11, Me-15
14*β*	2.46, br d, 1H (15.5)		H13, H14α		
15	1.06, s, 3H	26.6 (q)		H2, H11, H14α	H2, H11, H14*β*
16	0.94, s, 3H	16.1 (q)		H2, H6, H11	H3α*β*, H5*β*, H10
17	1.32, s, 3H	22.2 (q)		H6, H8α	H8α
18	2.39, m, 1H	31.0 (d)	Me-19, Me-20	H9α, Me-19, Me-20	Me-19, Me-20
19	1.10, d, 3H (6.5)	21.2 (q)	H18	H13, H18	Me-20
20	0.98, d, 3H (6.8)	22.7 (q)	H18	H11, H18	Me-19

[a] Chemical shift values are in ppm relative to TMS. Spectra were recorded at 25 °C. [b] ^{13}C NMR multiplicities were obtained from an Attached Proton Test (APT) experiment. [c] Protons correlated to carbon resonances in ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6, 8 Hz.

olefinic signals at δ 5.06 and 4.95 (each 1H, br s), together with its corresponding signal in the ^{13}C NMR spectrum at δ 112.4 (t). The relatively high field chemical shifts of H-9α*β* helped us to establish the position of the terminal double bond in **2** at C-7 (rather than at C-8) thus confirming the irregular "head to tail" isoprene configuration of this skeleton.[7] Many of the remaining NMR spectral features indicated no further differences between the structures of these compounds, implying that **2** was likely formed from an acid-catalyzed epoxide ring opening of **1**. The observed proton coupling constants between H-6 and the H_2-5α*β* protons [(axial−axial, 10.7 Hz) and (axial−equatorial, 4.9 Hz)] are consistent with the NOE data. Thus, NOESY correlations were observed between H-5*β* (δ_H 1.63) and H-3*β* (δ_H 2.53), which in turn showed a NOE to the H-10 signal at δ 1.49. Overall, these data confirmed that the proton in the C-10 position in compounds **1** and **2** is indeed *β*-oriented. An additional NOE interaction in **2** defined the cis relationship between the C-16 methyl group and the C-6 proton in ring A. In rings B and C, correlations between the methine proton H-11 and the methyl protons at C-1 and C-4 showed their respective proximities beneath the molecule.

The new diterpene, epipolol (**3**), was isolated as a colorless viscous oil. The high-resolution mass measurement established the molecular formula as $C_{20}H_{32}O_2$. The IR spectrum contained a strong hydroxyl band at 3362 cm^{-1} and bands at 1629 and 1260 cm^{-1}, both of which must be due to olefin and epoxide functionalities. The ^{13}C NMR spectrum contained the expected 20 signals with 31 attached protons, which required that there be one hydroxyl group in the compound. The ^{13}C NMR contained signals due to a trisubstituted olefin [δ 157.2 (s) and 120.7 (d)], a trisubstituted epoxide [δ 60.4 (d) and 60.2 (s)], and a secondary alcohol [δ 72.6 (d)]. Since the molecular formula requires five unsaturation equivalents, epipolol (**3**) must be tricarbocyclic. Thus, it quickly became apparent that **3** contained a hydroxyl group in place of the ketone functionality in epipolone (**1**). The location of the secondary alcohol at C-2 was determined from the HMBC correlations between the carbon signal at δ 72.6 (d) and the H_2-3 and H_3-15 signals. In the ^{1}H NMR spectrum (Table 3), the H-2 signal at δ 3.80 (dd, 1H, J = 5.3, 12.0 Hz) was coupled to the H_2-3 signals at δ 1.52 (dd, 1H, J = 5.3, 12.6 Hz) and 1.39 (br dd, 1H, J = 12.0, 12.6 Hz) which in turn showed no further coupling. The relative stereochemistry at C-2 in epipolol (**3**) was clearly defined by the NOE correlations between the H-2 signal and the H-3α (δ_H 1.52), H_3-15 (δ_H 1.06), and H_3-16 (δ_H 0.94) signals. Further analysis of the NOESY spectrum (Table 3) revealed that the relative stereochemistry at C-1, C-4, C-6, C-7, C-10, and C-11 was the same in both **1** and **3**. It is interesting to note that changing the ketone functionality in **1** to a secondary hydroxyl group in **3** had a profound effect on the ^{1}H NMR chemical shifts of the H-10 and H-11 signals (Tables 1 and 3). Additional significant variations in the NMR data were the ^{13}C NMR chemical shifts of C-1 (δ 54.5 versus 48.7), C-3 (δ 54.0 versus 46.7), C-14 (δ 42.6 versus 35.6), and C-15 (δ 22.5 versus 26.6).

Three known terpene compounds were also identified in this study: reiswigin B (**4**), (+)-curcuphenol (**5**), and (+)-curcudiol (**6**).[4,5] Compounds **4**−**6** were isolated in large amounts in comparison to **1** and **3**. Diterpenes **1**−**3**, on the other hand, have complex structures as they incorporate an unusually substituted tricarbocyclic framework. Another interesting structural feature of this class of diterpenes is the cis B/C ring fusion.[8] Interestingly, compounds **1**−**3** share more than one analogy with the dolastane class of diterpenes, typically isolated from the organic extracts of algae, and the recently discovered guanacastane class of diterpenes isolated from endo-

(7) The ^{1}H and ^{13}C NMR chemical shifts of C-8 in isoepipolone (**2**) are indicative of the allylic nature of C-8 (Tables 1 and 2). This connection was supported by complementary $^3J_{CH}$ HMBC correlations from H_2-17α*β* to C-8.

phytic fungi (Figure 1).[9,10] Indeed, while the tricarbocyclic backbone present in dolastanes and guanacastanes is reminiscent of that found in the present compounds, the biogenesis of **1** and **3** appears to involve atypical terpenoid pathways resulting in irregular "head to tail" isoprene configurations.[11] In vitro antituberculosis screening of compounds **2**−**4** against *Mycobacterium tuberculosis* H37Rv at a concentration of 6.25 μg/mL showed no inhibitory activity. Compounds **1**−**3** also proved to be inactive as potential inhibitors of the cell cycle regulators cdc2/cyclin B kinase and cdc25 phosphatase.[12] At 500 μg/mL epipolone (**1**) and epipolol (**3**) did not display significant in vivo cytotoxicity in the brine shrimp lethality bioassay (BSLT).[13]

Experimental Section

General Experimental Procedures. Infrared spectra were recorded with a FT-IR spectrophotometer. ^{1}H and ^{13}C NMR spectral data and ^{1}H−^{1}H COSY, NOESY, APT, HMQC, and HMBC experiments were measured with a 300 MHz FT-NMR spectrometer. Column chromatography was performed on silica gel (35−75 mesh) or bonded C_{18} silica gel (35−75 mesh). TLC analyses were carried out using glass silica gel plates. All solvents used were either spectral grade or were distilled from glass prior to use. The percentage yield of each compound is based on the weight of the dry sponge MeOH−$CHCl_3$ extract.

Extraction and Isolation of Compounds 1 and 3 and Other Major Constituents. The sponge specimen was collected by scuba from shallow reef waters off Desecheo Island, Puerto Rico and frozen shortly after collection.[14] The dry animal (90 g) was cut into small pieces and extracted with 1:1 MeOH−$CHCl_3$ (6 × 1 L). The combined organic extracts were concentrated and the residue obtained (16.4 g) was partitioned between hexane (4 × 400 mL) and water (300 mL). The hexane extract (6.3 g) was chromatographed over silica gel (180 g) using 10% EtOAc in hexane as eluant. Fractions were pooled based on their TLC and NMR profile to yield sixteen primary fractions, denoted as I−XVI. Fraction V (173 mg; 1.1% yield) was identified as the known sesquiterpene (+)-curcuphenol (**5**).[5] Fraction VII (925 mg) was submitted to another purification on silica gel (28 g) column using $CHCl_3$ as eluant, achieving pure reiswigin B (**4**) (555 mg; 3.4% yield).[4] Fraction IX (163 mg) was rechromatographed over silica gel (5 g) using $CHCl_3$ to obtain pure epipolone (**1**) (6.6 mg; 4.0×10^{-2} % yield). Fraction XI (966 mg) was purified by column chromatography on silica gel (29 g) using 1% 2-propanol in $CHCl_3$ to afford several fractions. Purification of the individual fractions was achieved by silica gel column chromatography eluting with 20% EtOAc in hexane and C_{18} reversed-phase silica gel using 10% water in MeOH to give the known sesquiterpene (+)-curcudiol (**6**) (446 mg; 2.7% yield)[5] and epipolol (**3**) (8.5 mg; 5.2×10^{-2} % yield), respectively.

Epipolone (1): colorless oil; $[\alpha]^{24}_D$ −6.7° (*c* 0.4, $CHCl_3$); IR (film) 3055, 1696, 1633, 1258, 1148, 1060 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HREI-MS *m*/*z* [M$^+$] calcd for $C_{20}H_{30}O_2$ 302.2246, found 302.2243 (33), 284.2143 (11, $C_{20}H_{26}O$), 259.1695 (19, $C_{17}H_{23}O_2$), 241.1611 (13, $C_{17}H_{21}O$), 150.1041 (28, $C_{10}H_{14}O$), 122.1097 (37, C_9H_{14}), 107.0847 (52, C_8H_{11}), 81.0687 (61, C_6H_9), 69.0716 (100, C_5H_9).

Random Decomposition of Epipolone (1) To Yield Isoepipolone (2). A solution of epipolone (**1**) (6.6 mg, 0.022 mmol) in $CDCl_3$ was kept inside a NMR tube at 25 °C for 1 week. The ^{1}H NMR spectrum and TLC analysis of the solution indicated that complete decomposition of **1** had slowly occurred. Purification by silica gel (1 g) column chromatography using 20% EtOAc in hexane as eluant yielded pure isoepipolone (**2**, 3.5 mg, 53% yield).

Isoepipolone (2): colorless oil; $[\alpha]^{24}_D$ −14.1° (*c* 1.6, $CHCl_3$); IR (film) 3226, 2922, 1700, 1262, 1097, 1017 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 2); HREI-MS *m*/*z* [M$^+$] calcd for $C_{20}H_{30}O_2$ 302.2246, found 302.2242 (9), 284.2128 (36, $C_{20}H_{28}O$), 241.1601 (23, $C_{17}H_{21}O$), 150.1037 (31, $C_{10}H_{14}O$), 122.1097 (53, C_9H_{14}), 107.0843 (100, C_8H_{11}), 91.0547 (57, C_7H_7), 69.0704 (51, C_5H_9).

Epipolol (3): colorless oil; $[\alpha]^{24}_D$ +16.4° (*c* 1.8, $CHCl_3$); IR (film) 3362, 3050, 1629, 1260, 1093, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 3); HREI-MS *m*/*z* [M$^+$] calcd for $C_{20}H_{32}O_2$ 304.2402, found 304.2404 (3), 286.2299 (27, $C_{20}H_{30}O$), 271.2062 (23, $C_{19}H_{27}O$), 243.1751 (25, $C_{17}H_{23}O$), 147.1173 (47, $C_{11}H_{15}$), 121.1017 (62, C_9H_{13}), 119.0860 (73, C_9H_{11}), 107.0863 (97, C_8H_{11}), 83.0491 (87, C_5H_7O), 81.0699 (72, C_6H_9), 55.0560 (100, C_4H_7).

Reiswigin B (4), (+)-Curcuphenol (5), and (+)-Curcudiol (6). The $[\alpha]_D$, ν_{max}, λ_{max}, ^{1}H and ^{13}C NMR, and HREIMS were identical in all respects to those previously reported.[4,5]

Acknowledgment. HREI mass spectral determinations were performed by Rául Blanco from the Material Characterization Center (MCC) of the University of Puerto Rico. Janet Figueroa recorded the FT-IR experiments. We thank Dr. Robert C. Reynolds and the U.S. Tuberculosis Facility (TAACF) for the antimycobacterial data of isoepipolone (**2**), epipolol (**3**), and reiswigin B (**4**); Dr. Laurent Meijer (C.N.R.S., France) for assaying compounds **1**−**3** as chemical inhibitors of the cyclin-dependent kinases; and Dr. Ricardo Guerrero (UPR-RCM) and Ileana I. Rodríguez (UPR-Río Piedras) for the in vivo cytotoxicity data of **1** and **3**. Support for this research was kindly provided by the NIH-MBRS (Grant S06RR08102-17) program of the University of Puerto Rico at Río Piedras.

Supporting Information Available: ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HMBC, and NOESY spectral data for compounds **1**−**3** and HMQC spectra for compounds **2** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0104734

(8) Usually, diterpenes containing various rings of different sizes have the five-membered carbocyclic ring trans fused to a larger ring, as in the cyathanes, verrucosanes, dolastanes, and dolabellanes; see: (a) Ayer, W. A.; Browne, L. M. *Tetrahedron* **1981**, *37*, 2199−2248. (b) Takaoka, D.; Matsuo, A.; Nakayama, M.; Hayashi, S. *Phytochemistry* **1983**, *22*, 1653−1655. (c) Rodríguez, A. D.; González, E.; Ramírez, C. *Tetrahedron* **1998**, *54*, 11683−11729.

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(14) Shortly before the publication of this manuscript, we learned that the sponge *Epipolasis reiswigi* had been synonymized with *Myrmekioderma styx* (de Laubenfels). Compounds with the same tricarbocyclic skeleton as **1**−**3** have been previously reported from deep water samples of *M. styx* collected near Venezuela and Jamaica, and from the Australian sponge *Higginsia* sp.; see: (a) Sennett, S. H.; Pomponi, S. A.; Wright, A. E. *J. Nat. Prod.* **1992**, *55*, 1421−1429. (b) Green, D.; Goldberg, I.; Stein, Z.; Ilan, M.; Kashman, Y. *Nat. Prod. Lett.* **1992**, *1*, 193−199. (c) Cassidy, M. P.; Ghisalberti, E. L.; Jefferies, P. R.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1985**, *38*, 1187−1195. We thank Dr. Amy E. Wright (HBOI) for fruitful discussions.