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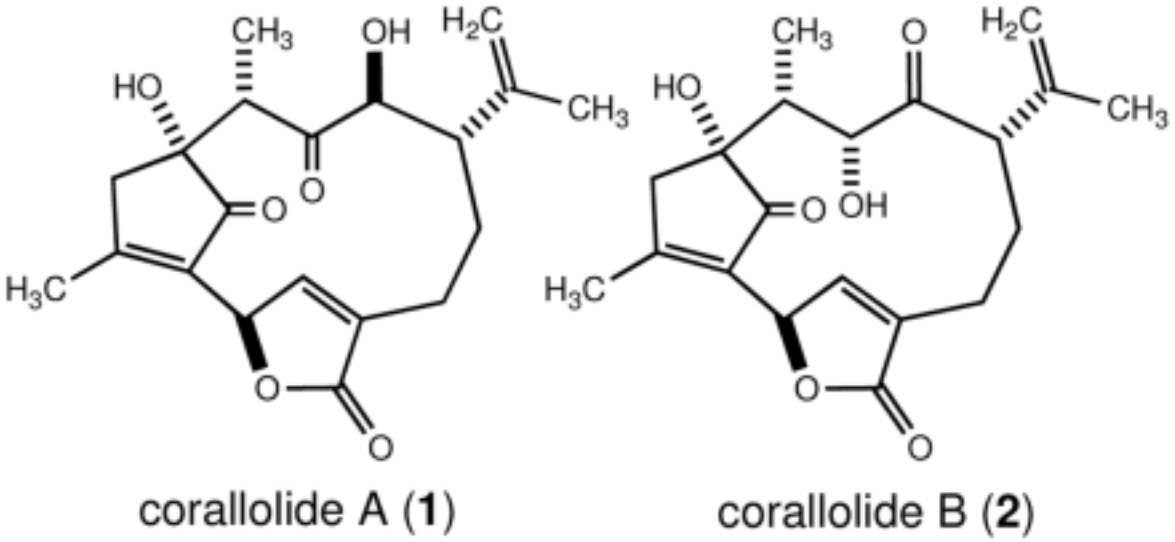
Corallolides A and B: Bioactive Diterpenes Featuring a Novel Carbon Skeleton

Claudia A. Ospina and **Abimael D. Rodríguez***
Department of Chemistry, University of Puerto Rico, P.O. Box 23346, U.P.R. Station, San Juan, PR 00931-3346

Abstract



Corallolides A (1) and B (2) are naturally occurring diterpenes isolated from the Caribbean gorgonian octocoral *Pseudopterogorgia bipinnata* collected near Providencia Island, Colombia. Their tricyclic structures are based on a uniquely substituted bicyclo[9.2.1]tetradecane ring system that was established through detailed spectroscopic analysis. Compounds 1 and 2 were shown to exhibit anti-parasitic and anti-tuberculosis activity, respectively.

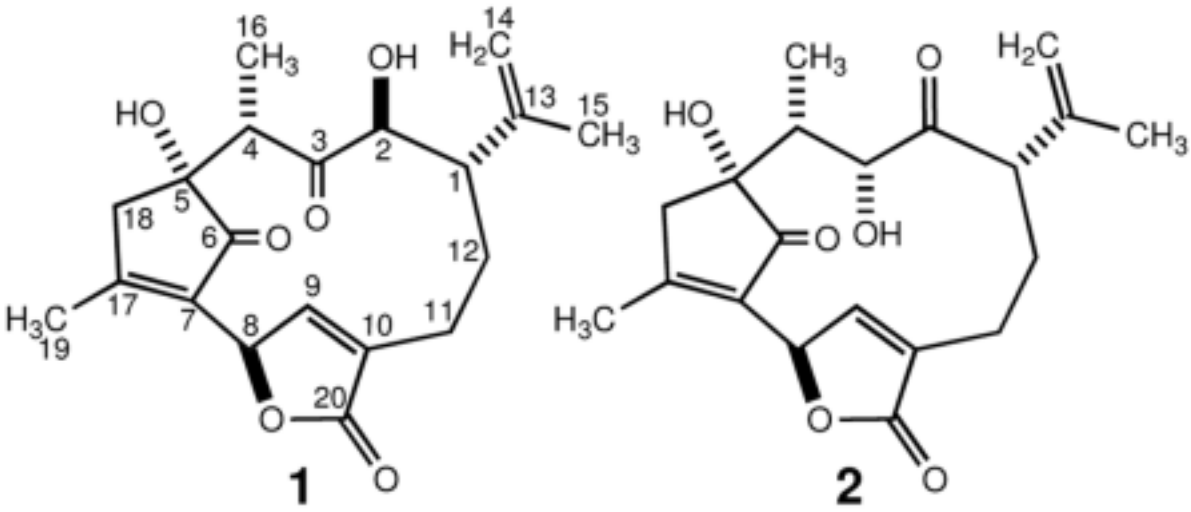
For nearly four decades, investigations into the natural products chemistry of Caribbean gorgonian octocorals of the genus *Pseudopterogorgia* have yielded a plethora of diterpenoids of diverse molecular architectures that endow them with remarkable biological activities.[1] The gorgonian species *P. bipinnata*, *P. kallos*, *P. acerosa*, *P. americana*, and *P. elisabethae* are particularly noteworthy as they alone appear to account for the production of the vast majority of these fascinating natural products.[2] Recently, the groups of Mulzer, Trauner, and Kerr have highlighted the ever increasing number of diterpenoid skeletal classes produced by these chemically prolific *Pseudopterogorgia* species. Furthermore, many of the metabolites engendered by these animals are highly likely to experience subsequent rearrangement to yield

abrodriguez@uprrp.edu.

Supporting Information Available: Figure 2, Schemes 1A and 1B, copies of NMR spectra of **1** and **2**, and experimental details for the extraction of *P. bipinnata* and the computational methods. This material is available free of charge via the Internet at http://pubs.acs.org.

even more perplexing structural variants.[3] The chemical investigation of these five octocoral species has led to important pharmacological advances which in turn have spurred novel synthetic methodology and inspired many chemists and biochemists to speculate on the plausible biogenetic relationships among the diverse skeletal families of diterpenes that are routinely co-isolated from them.

As part of our continuing interest in the natural products chemistry of Caribbean gorgonian octocorals and their potential utilization in the development of novel anti-infective and anti-cancer drugs, we have previously reported on several pseudopterane lactones from a Southwestern Caribbean specimen of *P. bipinnata*.[4] From the same specimen, we now report the isolation and structure elucidation of two isomeric diterpenes which feature a uniquely substituted bicyclo[9.2.1]tetradecane ring system. These compounds, which have been trivially named corallolides A (**1**) and B (**2**), feature a twelve-membered macrocycle that appears to be linked biosynthetically to the pseudopterane class of diterpenes through additional C–C bond formation leading to an intriguing bridged ring system. Their molecular structures were established by analysis of 1D and 2D NMR, IR, UV, and high-resolution mass spectral data. Biological screening of metabolites **1** and **2** revealed that they are bestowed with anti-tubercular and anti-parasitic activity.



A detailed scheme for the extraction of the dry gorgonian specimen (0.11 kg) has been provided as Supporting Information. The crude $CHCl_3$ extract (4.1 g) was fractionated over silica gel (150 g) with a 99:1 mixture of $CHCl_3$/MeOH to give 22 fractions, denoted I–XXII. Fractions IX and X were combined (126 mg) and chromatographed over silica gel (7 g) using 1% acetone in $CHCl_3$ leading to 7 secondary fractions (A–G). Further purification of sub-fraction D (37 mg) by normal-phase HPLC afforded pure corallolide A (**1**) (4.5 mg, 4.1×10^{-3}% yield) along with corallolide B (**2**) (5.0 mg, 4.5×10^{-3}% yield) (dry gorgonian weight basis).

The molecular formula of corallolide A (**1**)[5] was assigned as $C_{20}H_{24}O_6$ on the basis of high-resolution mass measurement of the $[M + Na]^+$ ion at *m/z* 383.1483 and overall NMR information indicating the presence of nine degrees of unsaturation in the molecule. The IR data for **1** indicated the presence of hydroxyl (3467 cm^{-1}), carbonyl (1756 and 1706 cm^{-1}) and olefin (1628 cm^{-1}) bands. The carbonyl bands at 1756 and 1706 cm^{-1} showed comparable intensity suggesting the presence in **1** of a conjugated γ-lactone and a 2-cyclopentenone ring, respectively. The latter contention was supported by strong UV absorptions at λ_{max} 214 nm (ε 14 200) and 235 nm (ε 12 300). Although **1** was soluble in $CDCl_3$, the ^1H and ^{13}C NMR

signals in this solvent were broad and poorly dispersed. Consequently, 1D and 2D NMR data were gathered in CD_3OD. The ^{13}C NMR spectrum (75 MHz, CD_3OD) of **1** showed 20 resolved resonances (Table 1). Six olefinic [δ 174.0 (C), 153.8 (CH), 146.1 (C), 137.0 (C), 132.6 (C), 114.7 (CH_2)] and three carbonyl [δ 212.9 (C), 208.0 (C), 174.8 (C)] resonances in the ^{13}C NMR spectrum accounted for six sites of unsaturation. Therefore, the remaining sites of unsaturation required by the molecular formula had to be explained by the presence of three rings. HMQC and DEPT-135 data showed that 22 of the 24 hydrogen atoms were attached to carbons (3 × CH_3, 4 × CH_2, 5 × CH); therefore, compound **1** had to have 2 OH groups.

Four partial structures (**A**–**D**) were deduced from extensive analyses of the 2D NMR data of **1** including COSY, NOESY, HMQC, and HMBC spectra in CD_3OD (Figure 1). The COSY, NOESY, and HMBC correlations for corallolide A (**1**) are presented in Table 1.

Connectivities from C11 to C2 in substructure **A**, including additional connectivities to an isopropylene group attached to C1, were inferred from the ^1H–^1H COSY cross-peaks (Table 1). HMBC correlations of C1 (δ_C 47.4) with H2 (δ_H 3.95), H_2-14 (δ_H 4.73 and 4.75), and H_3-15 (δ_H 1.60), and long-range couplings of C13 (δ_C 146.1) with H2 and H_3-15 allowed assignment of the structure for unit **A**. Partial structure **B**, an isolated –CH–CH_3 spin system (C4 and C16), was clearly revealed by the ^1H NMR, COSY, and HMQC spectra. The chemical shifts of H4 (δ_H 2.68) and C4 (δ_C 48.6) (as well as the multiplicities and coupling constants of all the protons) allowed us to further link units **A** and **B** through C3 (δ_C 212.9). This was supported by complementary HMBC correlations between C3 and protons H2, H4, and H_3-16.

The presence of a butenolide moiety in the molecule (unit **C**) was deduced from the COSY and HMBC spectra, the proton and carbon chemical shifts at positions C8–C10 and C20, together with IR and UV data (*vide supra*). Long-range couplings between H9 (δ_H 8.20) and C8, C10, and C20 were consistent with partial structure **C**. Furthermore, substructures **A** and **C** were confidently linked by the $^3J_{CH}$ correlations between H_2-11 (δ_H 2.53) and C20 (δ_C 174.8). Applying these combined NMR methods resulted in the unambiguous assignment of all the protons and carbons in units **A**–**C**.

For partial structure **D** (C5–C7 and C17–C19), connectivities from C17 through C19 were clearly revealed by the ^1H–^1H COSY and HMQC spectra. It was, however, difficult to obtain unambiguous evidence for connecting H_2-18 (an AB system at δ_H 2.60 and 2.81) to other spin systems. Notwithstanding, the connectivity of unit **D** was completed by careful analysis of the proton and carbon chemical shifts at positions C5, C6, C7, C17, C18 and C19, and by HMBC correlations for H18β/C5/C6/C7, H18αβ/C17, and H_3-19/C7/C17/C18. Thus, unit **D** was formulated as a 2,3,5,5-tetrasubstituted 2-cyclopentenone moiety, a contention that was in full agreement with the IR and UV spectra recorded for **1**. The pivotal ^{13}C NMR resonances at δ_C 208.0 (C), 174.0 (C), and 137.0 (C) were thus ascribed, respectively, to C6, C17, and C7 of the 2-cyclopentenone ring system. Confirmation of the substitution pattern of the latter system as well as its connectivity to the other ring systems found in **1** came from additional HMBC and TOCSY correlation data. Critically, HMBC cross-peaks between the hydroxyl-bearing quaternary carbon C5 (δ_C 83.6) and protons H4, H_3-16, and H18β allowed the attachment of units **B** and **D** through C4 and C5, respectively. At this point, the link between units **D** and **C** through C7 and C8, allowed the complete planar structure for **1** to be assigned. Weak but diagnostic homoallylic couplings (^5J) between the protons of C18 and C19 with lactone methine H8 confirmed the latter connectivity.[6] Interestingly, such linkage, which renders H8 bisallylic (δ_H 5.93), imposes upon the geometry of **1** in such a manner that H9, which lies close to the C=O bond of unit **D**, cuts into the deshielding cone of the induced magnetic field causing H9 to be unusually deshielded (δ_H 8.20). These analyses confidently established the gross structure shown for **1**.

The relative configurations (1*S**,2*S**,4*S**,5*R**,8*R**) for the five stereocenters about the tricyclic framework of corallolide A were assigned primarily on the basis of ^{1}H NMR coupling constants and NOESY data supported by distance calculations (McSpartan'04 Program). As $^{3}J_{H1\text{-}H2}$ was only 4.8 Hz, a refutable *trans*-relationship between these protons was proposed.[4] This assignment, however, was firmly established by the strong NOESY correlations of H1 (β-orientation in planar conformation) and H4, plus the conspicuous absence of a NOE between H2 and H4 (calcd inter-proton distance = 3.2 Å), thus placing the isopropenyl side chain at C1 and H_3-16 in the same α-plane. Synchronous NOESY cross-peaks between H2 and pivotal protons H9 and H12α further supported these assignments. After calculating the conformer distribution of each possible stereomeric structure of **1** at C5 and C8, the coincident NOESY correlations of H2/H9, H4/H18β, H_3-16/H18β, and H9/H12α were consistent only with structure **1** (Figure 2*cf* Supporting Information). Only in this stereoisomer, which also possessed the lowest calculated energy (312 kJmol^{-1}), the intramolecular distances for the latter protons were calculated to be within 2.6–2.0 Å.

The structural characterization of corallolide B (**2**) was carried out in an analogous manner.[7] The HRESIMS and ^{13}C NMR data for **2** were consistent with a molecular formula of $C_{20}H_{24}O_6$. A side-by-side comparison between the NMR spectra of isomers **1** and **2** rapidly pinpointed their structural similarities. Nevertheless, some major differences between these compounds were observed in the ^{13}C NMR spectra: the signals ascribed to C1–C4 and C16 shifted from δ_C 47.4 (CH), 78.4 (CH), 212.9 (C), 48.6 (CH), and 13.2 (CH_3) in corallolide A (**1**) to 50.5 (CH), 209.8 (C), 73.1 (CH), 41.6 (CH), and 7.1 (CH_3) in **2**, respectively. Placement in corallolide B of a carbonyl at C2 and a hydroxyl (in the α-orientation) at C3 would account for these spectral differences. Consideration of ^{1}H and ^{13}C NMR data as well as COSY, NOESY, and HMBC data allowed the complete structure to be assigned as **2** and led to the unambiguous assignment of all the protons and carbons as listed in Table 2. The NOEs and the small coupling constant between H3 and H4 ($J_{H3/H4} < 1$ Hz), which require a dihedral angle close to 90° (calcd θ = −88.2°), correlated with the lowest energy conformer of a model representing the relative stereochemistry shown in structure **2**.

The co-ocurrence of compounds **1** and **2** with various pseudopterane lactones[4] within *P. bipinnata* raises the possibility that the corallolides represent a further modification of an existing metabolite, thus suggesting the biogenetic pathway shown in Scheme 1. We suggest the semisystematic name "corallolane" to define this new carbon skeleton and a numbering system that preserves the C1–C20 numbering of the pseudopterane skeleton.[8]

In our primary *in vitro* anti-tuberculosis assay (at 128 μg/mL), compound **2** inhibited growth of *Mycobacterium tuberculosis* by 95%, but was inactive against the malaria parasite *Plasmodium falciparum*. Compound **1**, however, displayed strong antimalarial activity (IC_{50} = 10 μg/mL).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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4. (a) Marrero J, Ospina CA, Rodríguez AD, Baran P, Zhao H, Franzblau SG, Ortega-Barria E. Tetrahedron 2006;62:6998–7008. (b) Ospina CA, Rodríguez AD, Zhao H, Raptis RG. Tetrahedron Lett 2007;48:7520–7523.
5. Corallolide A (**1**): colorless oil; $[\alpha]^{20}_{D}$–12 (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3467, 3098, 3077, 2941, 1756, 1706, 1628, 1442, 1379, 1332, 1111, 1068, 1039, 752 cm^{-1}; UV (MeOH) λ_{max} 214 (ε 14 200), 235 (ε 12 300), 278 (ε 1900) nm; HRESIMS *m/z* $[M + Na]^+$ 383.1483 (calcd for $C_{20}H_{24}O_6Na$, 383.1471).
6. In some alkenes coupling can occur between the C–H σ bonds on either side of the double bond. This type of coupling is generally very small or even nonexistent in most molecules, but it sometimes appears in NMR spectra; see: Jackman LM, Sternhell S. Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry (2) 2. Pergamon PressNew York1969:316–328.328
7. Corallolide B (**2**): colorless oil; $[\alpha]^{20}_{D}$–98 (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3458, 3084, 2929, 1756, 1707, 1629, 1442, 1379, 1336, 1204, 1070, 758 cm^{-1}; UV (MeOH) λ_{max} 208 (ε 8100), 234 (ε 6400), 279 (ε 900) nm; HRESIMS *m/z* $[M + Na]^+$ 383.1483 (calcd for $C_{20}H_{24}O_6Na$, 383.1471).
8. Erythrolide K, a diterpene isolated from the gorgonian coral *Erythropodium caribaeorum*, is based on a bicyclo[9.2.1]tetradecane ring system. Notwithstanding, the alkylation/substitution pattern about the corallolane skeleton is unprecedented in Nature; see: Banjoo D, Maxwell AR, Mootoo BS, Lough AJ, McLean S, Reynolds WF. Tetrahedron Lett 1998;39:1469–1472.1472

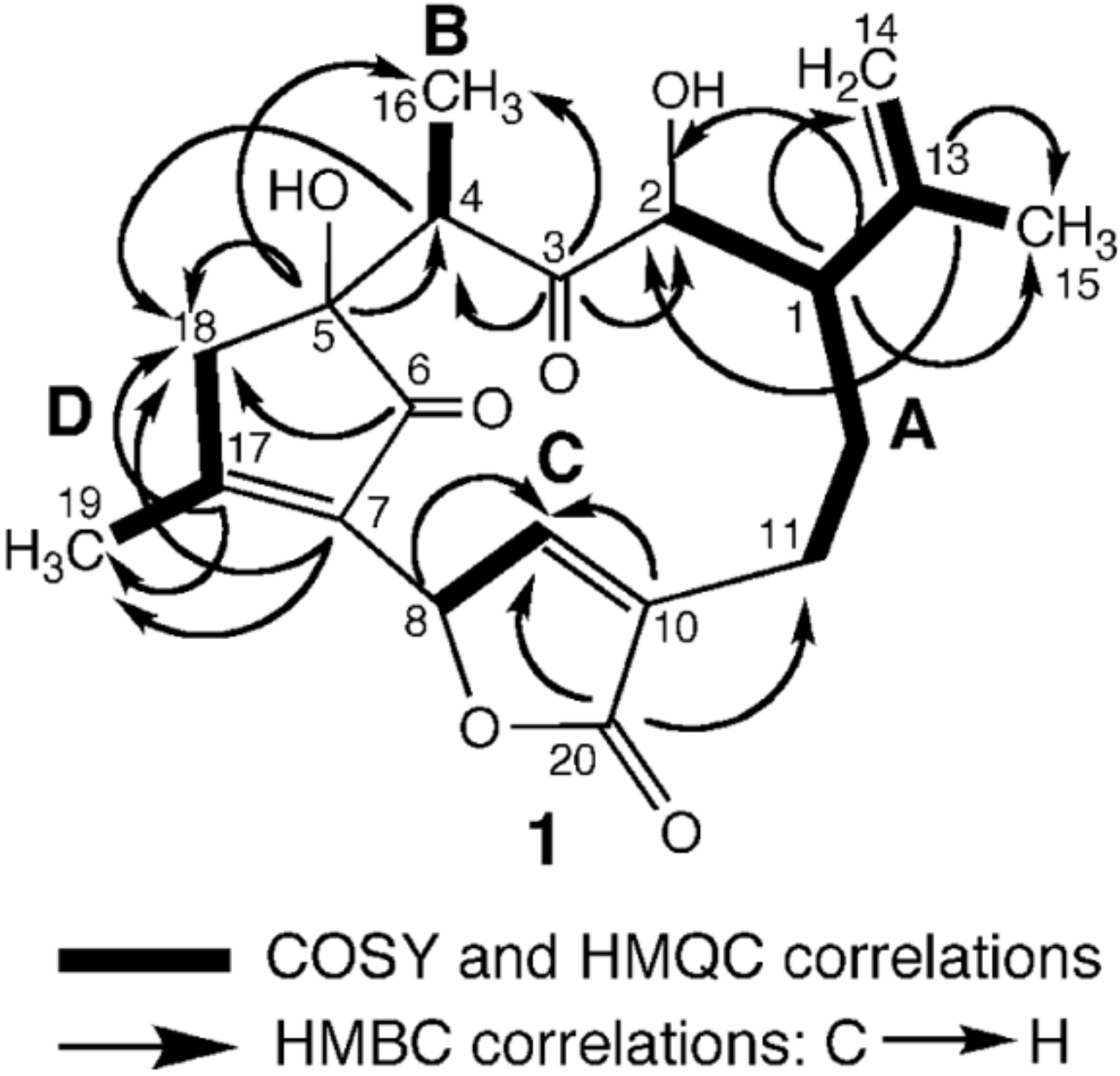

Figure 1.
Partial structures for corallolide A (**1**) generated from $^{1}H–^{1}H$ COSY, HMQC, and HMBC spectral data.

Table 1

^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H—^{1}H COSY, NOESY, and HMBC spectral data for corallolide A (**1**)[a]

position	δ_H, mult, intgt (*J*, Hz)	δ_C (mult)[b]	^{1}H–^{1}H COSY	NOESY	HMBC[c]
1	2.04, br m, 1H	47.4 (CH)	H2, H12αβ	H4, H14αβ	H2, H14αβ, H_3-15
2	3.95, d, 1H (4.8)	78.4 (CH)	H1	H9, H12α	
3		212.9 (C)			H2, H4, H_3-16
4	2.68, q, 1H (6.8)	48.6 (CH)	H_3-16	H1, H14αβ, H_3-16, H18β	H_3-16, H18β
5		83.6 (C)			H4, H_3-16, H18β
6		208.0 (C)			H18β
7		137.0 (C)			H18β, H_3-19
8	5.93, br s, 1H	77.1 (CH)	H9, H18α, H_3-19	H9, H_3-19	H9
9	8.20, br s, 1H	153.8 (CH)	H8	H2, H8, H11α, H12α	
10		132.6 (C)			H9
11αβ	2.53, br m, 2H	22.5 (CH_2)	H12αβ	H9	
12α	1.71, br m, 1H	27.4 (CH_2)	H1, H11αβ, H12β	H2, H9	H1
12β	2.00, br m, 1H		H1, H11αβ, H12α		
13		146.1 (C)			H2, H_3-15
14α	4.73, br s, 1H	114.7 (CH_2)	H14β, H_3-15	H4, H14β, H_3-15	H_3-15
14β	4.75, br s, 1H		H14α, H_3-15	H1, H4, H14α	
15	1.60, br s, 3H	21.1 (CH_3)	H14αβ	H14αβ	H14αβ
16	1.16, d, 3H (6.8)	13.2 (CH_3)	H4	H4, H18β	H4
17		174.0 (C)			H18αβ, H_3-19
18α	2.60, br d, 1H (17.4)	46.7 (CH_2)	H8, H18β, H_3-19	H18β	H_3-19
18β	2.81, br d, 1H (17.4)		H18α	H4, H_3-16, H18α	
19	2.29, br s, 3H	19.1 (CH_3)	H8, H18α	H8	
20		174.8 (C)			H9, H11αβ

[a] Spectra were recorded in CD_3OD at 25 °C. Chemical shift values are in parts per million relative to the residual CH_3OH (3.30 ppm) or CD_3OD (49.0 ppm) signals. Assignments were aided by 2D NMR experiments, spin-splitting patterns, number of attached protons, and chemical shift values.

[b] ^{13}C NMR multiplicities were obtained from a DEPT-135 experiment.

[c] Protons correlated to carbon resonances in the ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6 and 8 Hz.

Table 2

^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), ^{1}H—^{1}H COSY, NOESY, and HMBC spectral data for corallolide B (**2**)[a]

position	δ_H, mult, intgt (*J*, Hz)	δ_C (mult)[b]	^{1}H–^{1}H COSY	NOESY	HMBC[c]
1	2.74, br d, 1H (10.4)	50.5 (CH)	H12β	H3, H4, H14αβ	H14αβ, H_3-15
2		209.8 (C)			H1, H3
3	4.44, s, 1H	73.1 (CH)		H1, H4, H9, H12α	H_3-16
4	1.78, br q (6.9)	41.6 (CH)	H_3-16	H1, H3, H_3-16	H3, H_3-16
5		84.3 (C)			H3, H_3-16
6		208.1 (C)			H18α
7		135.7 (C)			H18α, H_3-19
8	5.89, br s, 1H	76.1 (CH)	H9, H18αβ, H_3-19	H9	H9
9	8.09, br s, 1H	150.5 (CH)	H8	H3, H8, H12αβ	
10		133.6 (C)			H9, H11αβ
11αβ	2.42, br m, 2H	20.8 (CH_2)	H12αβ		H1, H12α
12αβ	1.54, br m, 1H; 2.17, br m, 1H	31.6 (CH_2)	H1, H11αβ	H3, H9	H1
13		138.2 (C)			H1, H_3-15
14αβ	5.08, br s, 1H; 4.80, br s, 1H	115.3 (CH_2)	H_3-15	H1, H_3-15	H1, H_3-15
15	1.70, br s, 3H	21.8 (CH_3)	H14αβ	H14α	H1, H14αβ
16	0.88, d, 3H (6.9)	7.1 (CH_3)	H4	H4, H18α	H3, H4
17		169.1 (C)			H18α, H_3-19
18αβ	2.64, br d, 1H (17.3); 2.77, br d, 1H (17.3)	44.3 (CH_2)	H8	H_3-16	H_3-19
19	2.33, br s, 3H	19.4 (CH_3)	H8		
20		174.0 (C)[d]			H9

[a] Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in parts per million relative to the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals. Assignments were aided by 2D NMR experiments, spin-splitting patterns, number of attached protons, and chemical shift values.

[b] ^{13}C NMR multiplicities were obtained from a DEPT-135 experiment.

[c] Protons correlated to carbon resonances in the ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6 and 8 Hz.

[d] Due to its low intensity the chemical shift value of this peak was carefully estimated from HMBC experiments.