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Plant-like Cadinane Sesquiterpenes from an Actinobacterial Mangrove Endophyte

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Abstract

Cadinanes are typical plant sesquiterpenes with a broad range of biological functions. We report the isolation of three cadinanes (**1–3**) from a bacterial endophyte (*Streptomyces* sp.) of the mangrove plant *Bruguiera gymnorrhiza*. The structures of two new cadinenens, (+)-11-hydroxy-epicubenol (**1**) and (+)-12-hydroxy-epicubenol (**2**) were elucidated by NMR and mass spectrometry. The bacterial product (+)-11-hydroxy-epicubenol was elucidated to be an enantiomer of the plant product pubinernoid C. (+)-12-Hydroxy-epicubenol was established as a diastereomer of the basidiomycete product trichapargin A. In addition, a crystal structure analysis corroborated the structure and configuration of 5,11-epoxy-10-cadinanol (**3**), a cadinane cycloether initially described as a natural product from liverworth. The discovery of oxygenated cadinanes from a bacterial endophyte may set the basis for the production of cadinanes by bacterial fermentation.

KEYWORDS: NMR; 2D NMR; cadinane; endophyte; sesquiterpene; *Streptomyces*; terpene; epicubenol

INTRODUCTION

Cadinanes (examples in Figure 1) constitute a large family of plant and fungal terpenes with characteristic decaline (cadinane, or cadalane) scaffolds that result from the C-1/C-6, C-5/C-10 cyclization of farnesyl pyrophosphate. Depending on their architectures and substitution patterns, cadinane sesquiterpenoids play diverse biological functions or have pharmaceutically relevant bioactivities.

For example, γ -cadinene, (+)- δ -cadinene, and T-muurolol are volatiles that contribute to the bouquets of many plants, such as hops and *Tagetes*.^[1] Cadinene is the precursor of the herbivore defense compound gossypol in cotton.^[2] T-Cadinol is a larvicidal and antimycobacterial agent found in the bark of the Peruvian plant *Swartzia polyphylla*.^[3] α -Cadinol from *Taiwania cryptomerioides*^[4] and (–)-(5*R*, 6*R*, 7*S*, 9*R*, 10*S*)-cadinan-3-ene-6,7-diol from *Eupatorium adenophorum* leaves^[5] are cytotoxic against various cancer cell lines, and polygousmic acid inhibits the growth of drug-resistant *Escherichia coli* and *Staphylococcus aureus* (MRSA) strains.^[6] The most important seco-cadinane-derived drug is the antimalarial agent artemisinin.^[7] While artemisinin is traditionally obtained by extraction of *Artemisia* species, there is increasing interest in the semisynthesis of the drug from cadinane precursors such as artemisinic acid obtained by fermentation of recombinant yeast expressing plant genes.^[8]

Microbial sources of cadinanes would be valuable for synthetic biology approaches,^[9] yet reports on such microbial sesquiterpenes are extremely scarce. At the time this study was conducted, few examples were γ -cadinenes from termite-associated fungus *Termitomyces*^[10] and *Chitinophaga pinensis*,^[11] bacterial cadinene sesquiterpenes were an unspecified cadinene from *Streptomyces citreus*^[12] and (–)- δ -cadinene as well as (+)-T-muurolol, the antipodes of

the plant metabolites, (+)- δ -cadinene, and T-muurolol, were obtained by expression of cryptic sesquiterpene synthase from both *Streptomyces clavuligerus* and *Roseiflexus castenholzii*.^[11] ^[13] T-muurolol and oxygenated congeners have also been reported as natural products from a marine *Streptomyces* isolate.^[14] Here we report on the isolation and structure elucidation of three cadinane-type sesquiterpenes, from a bacterial endophyte residing in the stem of the mangrove plant *Bruguiera gymnorrhiza*.

RESULTS AND DISCUSSION

In the course of searching for new secondary metabolites from bacterial endophytes inhabiting mangrove trees,^[15] we noted that the associated microbes are prolific producers of terpenoids, including sesquiterpenes bacaryolanes^[16] and kandenols^[17] as well as indolosesquiterpenes.^[18] A closer inspection of the metabolic profile of a *Streptomyces* sp. (JMRC:ST027706) isolated from the stem of *Bruguiera gymnorrhiza* revealed a range of terpenoids in the nonpolar fractions. The discovery of rare bacterial caryolanes^[16] from this strain encouraged us to elucidate the fraction of other essential oil components. Therefore, we fractionated extracts of the fermentation broth (200 L) by chromatography on Amberchrom 161c resin LC, silica gel, and Sephadex LH-20 columns, yielding pure **1** (1.4 mg), **2** (4.3 mg), and **3** (7.9 mg).

The structures of **1–3** were elucidated by MS, NMR (Figure 2), and X-ray crystallography of **3** (Figure 3). The molecular formula of compound **1** was proposed to be C₁₅H₂₆O₂ (m/z [M + H]⁺ 239.2006, Δ -0.2 ppm). The ¹H NMR spectrum of **1** shows signals from one olefinic proton (H-5, δ 5.63), four methyl groups and signals from other aliphatic protons. The ¹³C NMR spectrum revealed 15 carbon signals indicating **1** as a sesquiterpene.

The COSY spectrum revealed key correlations between H-5 and H-6 (s, δ 1.84) and H-3 (δ 2.08), H-6 and H-7 (s, δ 1.35), and H-7 and H-8 (2H, δ 1.04, δ 1.74), and further correlations between H-2 and H-3, and H-8 and H-9, which indicated a decaline spin system. Further analysis of HSQC and HMBC data supported **1** as a cadinene-type sesquiterpene with a trans-fused cyclohexene ring. A relative lower chemical shift for C-3 (δ 26.6, trans >30 ppm) indicated a cis-decalin skeleton for **1**. A large coupling constant (11.1 Hz) between H-6 and H-7 suggested a trans-orientation between them. Furthermore, NOESY correlations (Figure 2) between H-6 and H-9b, H-7 and H-9a, Me-14 and H-9a, H-6 and H-10 led to a suggestion of **1** with either a cubenol (**4**) or epicubenol (**5**) skeleton. Notably, the ^{13}C NMR data of **1** (Table 1) are identical with those reported for pubinernoid C (**6**), an epi-cubenol type terpene isolated from the plant *Schisandra pubescens* var. *pubinervis*.^[19] Interestingly, this microbial 11-hydroxy-epicubenol (**1**) ($[\alpha]_D^{23}$ +40.7, MeOH) appeared to be an enantiomer of the plant product pubinernoid C ($[\alpha]_D^{19}$ -60.7, MeOH) deduced from the optical rotation.^[19]

Compound **2** appears to be a second new epi-cubenol. The molecular formula of compound **2** was proposed to be $\text{C}_{15}\text{H}_{26}\text{O}_2$ (m/z $[\text{M} + \text{H}]^+$ 239.2006, Δ -0.2 ppm). Comparison of NMR spectral data (Table 1) indicated that compound **2** shares the same epi-cubenol skeleton as **1**. For example, the chemical shifts of C-1, C-6 and C-14 were conserved between **1** and **2**. The large coupling constant (11.3 Hz) between H-6 and H-7 indicates a *trans*-orientation as in **1**. However, in the ^1H NMR spectrum one methyl singlet is absent, instead signals of one oxygen-bonding methylene (H-12, δ 3.83, 3.42) appeared. The location of this proton at C-13 was inferred from COSY correlations between H-11 (δ 1.98), H-12 and Me-13 (δ 0.94). HMBC correlations (Figure 2) between H-13 and C-7 (δ 47.8), C-11 (δ 36.1) and C-12 (δ 15.8) further confirmed this. Thus **2** is confirmed to have a 1,13-dihydroxy-4-cadinene scaffold. Previous reports on two different muurolene isomers revealed distinct ^{13}C NMR data for C-13 (δ 10.7 ppm for (+)-(1R,3R,6S,7S,11R)-3,12-dihydroxy- α -muurolene (**7**) and δ

15.6 ppm for (+)-(1R,3R,6S,7S,11S)-3,12-dihydroxy- α -muurolene (**8**).^[20] By comparison, the relative configuration at C-7 and C-11 for **2** was established as **7** by ¹³C NMR data (δ 15.8, C-13) accordingly. An isomer of **2** named as trichapargin A (**9**) was recently reported from the mushroom *Trichaptum pargamenum*.^[21] Here we name **2** as (+)-12-hydroxy-epicubenol.

Cubenols are a group of cadinene sesquiterpenes reported from cubeb pepper oil.^[22] Among the first reports of bacterial volatiles, e.g. geosmin from Actinobacteria, Geber reported epicubenol as a sweet-spicy volatile terpene from bacteria.^[23] Recently, heterologous expression of an (+)-epicubenol synthase in *Streptomyces* sp. led to the production of the bacterial (+)-epicubenol.^[24] Here we report two new epicubenol alcohol derivatives (+)-11-hydroxy-epicubenol (**1**), and (+)-12-hydroxy-epicubenol (**2**), from a wild bacterial strain.

Compound **3** was obtained as another terpene belonging to the cadinane family. In the ¹H NMR spectrum, no olefinic proton signals were observed. MS and NMR data of **3** (Table 1, Figure 2) showed that this compound resembled the cycloether 5,11-epoxy-10-cadinanol, which was first isolated from the liverwort *Ptychanthus striatus*.^[25] To rigorously elucidate the absolute configurations of **3**, we grew crystals of this compound and subjected them to x-ray crystallography. The crystal structure (Figure 3) confirmed the architecture and absolute configuration of **3**. We found that **3** exhibits also a positive optical rotation ($[\alpha]_D^{23} +16.8$, MeOH), confirming the same absolute configuration as reported for the plant metabolite.^[21] Although we have not noticed any pronounced antimicrobial or cytotoxic activities in a preliminary whole-cell screening of the cadinanes, it has been reported that the major metabolite (**3**) formed through this pathway is cytotoxic.^[26]

The co-occurrence of the three sesquiterpenes in the mangrove endophyte suggests that **1–3** share a common biosynthetic pathway (Scheme 1). A plausible precursor of all

congeners would be the 10-carbon ring-containing *cis*-germacradienyl cation. Since the double bonds in the *cis*-germacradienyl cation are unfavourably placed for a cyclization reaction, a 1,3-hydride shift would generate a new cadinyl cation, and subsequent deprotonation would yield δ -cadinene.^[27] Regiodivergent oxygenation and cyclization would lead to the formation of compounds **1–3**.

The bacterial biosynthesis of the plant-derived natural products **1** and **3** is intriguing and they are important additions to the currently known metabolic potential of endophytes.^[28] Cadinane sesquiterpenoids have been reported as metabolites of *Phomopsis cassiae*, an endophytic fungus associated with *Cassia spectabilis*.^[29] It is particularly remarkable that 5,11-epoxy-10-cadinanol (**3**) has also been isolated from *Perenniporia tephropora*, a fungal endophyte from *Taxus chinensis* var. *mairei*.^[26] Only recently, the same compound was detected as a metabolite of *Streptomyces anulatus* isolated from *Giraffa camelopardalis* feces.^[30]

CONCLUSIONS

In conclusion, we have reported the isolation of three cadinanes from a mangrove endophyte, two of which have previously been reported as plant metabolites. Consequently, two cadinane structures are new (**1** and **2**). Furthermore, we show the crystal structure of **3** and thus corroborate the proposed structure. Together with recently published, related studies our findings demonstrate that plant-associated microbes represent a potential source of cadinanes originally isolated from plants. Thus, endophytes may be harnessed for the production of valuable cadinane-derived pharmaceuticals by fermentation. From an ecological point of view, the discovery of cadinane-producing endophytes is intriguing

because endophytes might, at least in part, account to the production of terpenoid volatiles that could play roles in plants communication, and cytotoxic compounds like **3** could contribute to the defensive armory against herbivores.

EXPERIMENTAL

General IR spectra were recorded on a Bruker FT-IR (IFS 55) spectrometer. UV spectra were recorded on a Cary 1 Bio UV-visible spectrophotometer (Variant). Optical rotation was recorded on a Propol digital automatic polarimeter (Dr. Wolfgang Kernchen GmbH, Seelze, Germany). EI spectra were recorded on SSQ 710 (manufactured by Finnigan MAT). CI spectra were measured on Thermo Q-Exactive GC coupled to a GC-Chromatography. Open column chromatography was performed on silica gel 60 (Merck, 0.04–0.063 mm, 230–400 mesh ASTM) and Sephadex LH-20 (Pharmacia). TLC analysis was performed on silica gel plates (Sil G/UV254, 0.20 mm, Macherey-Nagel). Preparative HPLC was performed on a Waters HPLC system using a Nucleosil 100-5 C₁₈ column (5 μ m, 250 x 16 mm) with a UV detector. The NMR spectra were recorded on Bruker Avance DRX 500 and 600 NMR spectrometers equipped with 5 mm probes, at 300 K. Samples of **1** (1.4 mg), **2** (4.3 mg), **3** (7.9 mg) were each dissolved in 0.5 ml of CDCl₃ and transferred into a 5 mm NMR tube. The ¹H, ¹³C NMR (at 500 and 125 MHz, respectively) were measured for compounds **1** and **2**. Meanwhile, ¹H, and ¹³C NMR spectra for compound **3** were measured at 600 MHz and 150 MHz, respectively. Chemical shifts are given on the δ scale and referenced to CDCl₃ at 7.24 ppm for proton and 77.38 ppm for carbon. Coupling constants (*J*) are in Hertz. For the 2D experiments, including COSY, HSQC, HMBC, and NOESY, all data were acquired with 2048 $\times$ 128 (*t*₂ $\times$ *t*₁), 1024 $\times$ 192 (*t*₂ $\times$ *t*₁), 2048 $\times$ 192 (*t*₂ $\times$ *t*₁), and 2048 $\times$ 192 (*t*₂ $\times$ *t*₁) data points, respectively. The HMBC experiment used a long-range coupling constant of 10 Hz.

Compound **1**: for ¹H NMR experiment, pulse sequence zg30, 128 transients were acquired with a 1s relaxation delay using 65536 data points. The 90° pulse duration was 14 μ s, and the

spectral width was 10000.000Hz. Data matrix size after Fourier transformation was 131072.

The ^{13}C NMR spectrum pulse sequence udef1 were obtained with a spectral width of 29761.904 Hz using 19996 data points. The 90° pulse duration was 13 μs . The HSQC spectrum was recorded with $^1J(\text{CH}) = 140$ Hz. The NOESY mixing time was 1.5s.

Compound 2: for ^1H NMR experiment, pulse sequence zg30, 128 transients were acquired with a 1s relaxation delay using 65536 data points. The 90° pulse duration was 14 μs , and the spectral width was 10000.000Hz. Data matrix size after Fourier transformation was 131072.

The ^{13}C NMR spectrum pulse sequence udef1 was obtained with a spectral width of 29761.904 Hz using 65536 data points, the spectral width 29761.904 Hz. The HSQC spectrum was recorded with $^1J(\text{CH}) = 140$ Hz. The NOESY mixing time was 1.2s.

Compound 3: for ^1H NMR experiment, pulse sequence zg30, 64 transients were acquired with a 1 s relaxation delay using 65536 data points. The 90° pulse duration was 8 μs , and the spectral width was 12019.230 Hz. Data matrix size after Fourier transformation was 262144.

The ^{13}C NMR spectrum pulse sequence zgpg30, obtained with a spectral width of 36231.883 Hz using 65536 data points; The HSQC spectrum was recorded with $^1J(\text{CH}) = 145$ Hz; The NOESY mixing time was 1.5s.

Strain Isolation and Taxonomic Classification. JMRC:ST027706 was isolated from the stems of *Bruguiera gymnorrhiza*. Isolation and taxonomy can be found in the previous report.

^[16] The bacterium is stored at Hans-Knoell Institute.

Extraction and Isolation

The broth of a 200 L fermentation of *Streptomyces* sp. JMRC:ST027706, an endophyte isolated from stems of *Bruguiera gymnorrhiza*,^[16] was filtered and loaded onto an Amberchrom 161c resin LC column (200 $\times$ 20 cm, 6 L). Elution with a linear gradient of $\text{H}_2\text{O}/\text{CH}_3\text{OH}$ (from 30% to 100% v/v, flow rate 0.5 L min^{-1} , in 58 min) afforded seven

fractions (F1–F7). F1–F5 were combined and extracted by ethyl acetate and evaporated to afford the extract (8 g). It was firstly fractionated by silica gel column chromatography with a CH₂Cl₂/CH₃OH gradient to yield four fractions, A–D. Fraction A was firstly fractionated by a Sephadex LH-20 (CH₂Cl₂/50% CH₃OH) column, and six fractions (A1–A6) were obtained. Fraction A1 was further separated by a RP-C₁₈ column (CH₃OH/H₂O as gradient) to afford seventeen FA1–A1 to FA1–A23. FA1–A15 were separated by silica gel column chromatography (CH₂Cl₂/CH₃OH as gradient) to afford **3** (7.9 mg). FA1–A16 was separated by silica gel column chromatography (CH₂Cl₂/CH₃OH as gradient) to afford **1** (1.4 mg). FA1–A17 was separated by silica gel column chromatography (CH₂Cl₂/CH₃OH as gradient) to afford **2** (4.3 mg).

(+)-11-Hydroxy-epicubenol (1): colorless solid; $[\alpha]_D^{23}$ 40.7 (*c* 0.44, MeOH); IR (film) $\nu_{\max}$ 3433.9, 2915.3, 1699.3, 1652.8, 1558.4, 1540.6, 1506.8, 1456.9, 1373.6, 1283.1, 1153.6, 1081.1, 1023.1, 965.1, 925.0, 890.2, 854.2, 791.7, 668.4, 629.2 cm⁻¹; EIMS (14 eV) *m/z*: 238, 220, 205, 202, 194, 187, 177, 174, 163, 159, 147, 132, 120, 106, 94, 59, 32; HRCIMS *m/z* [M + H]⁺ 239.2006 (calculated for C₁₅H₂₇O₂, 239.2006, Δ0.2 ppm); NMR data see Table 1.

(+)-12-Hydroxy-epicubenol (2): colorless solid; $[\alpha]_D^{23}$ 0.4 (*c* 0.04, MeOH); EIMS (14 eV): 238, 221, 205, 190, 179, 170, 159, 149, 147, 134, 119, 110, 92, 85, 81, 32; HRCIMS: [M + H]⁺ 239.2006 (calculated for C₁₅H₂₇O₂, 239.2006, Δ 0.2 ppm); NMR data see Table 1.

5,11-Epoxy-10-cadinanol (3): colorless solid; $[\alpha]_D^{23}$ 16.8 (*c* 0.38, MeOH); IR (film) $\nu_{\max}$ 3407.1, 2922.3, 1716.3, 1453.9, 1375.5, 1363.5, 1317.8, 1253.4, 1191.6, 1162.5, 1142.6, 1127.3, 1100.1, 1081.6, 1055.6, 1019.3, 1100.1, 1081.6, 1055.6, 1019.3, 1000.6, 980.4,

938.0, 915.6, 875.7, 836.7, 794.7, 767.0, 751.7, 649.8, 636.5 cm^{-1} ; EIMS (14 eV) m/z : 238, 223, 212, 205, 196, 180, 162, 147, 137, 122, 106, 95, 32; NMR data see Table 1.

Cytotoxicity Assays

A modified propidium iodide assay was used to determine the cytotoxic activities of compounds **2** and **3** against 12 cell lines derived from solid human tumors. The test procedure has been described elsewhere.^[31] Cell lines tested were derived from patient tumors engrafted as a subcutaneously growing tumor in NMRI nu/nu mice or obtained from the American Type Culture Collection (Rockville, MD, USA), National Cancer Institute (Bethesda, MD, USA), or Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). Inhibitory concentrations are provided as 50% inhibition of cell growth (absolute IC_{50} , determined by two-point-curve-fit after plotting compound concentration versus fluorescence intensity). Neither **2** nor **3** exhibited cytotoxic activities ($\text{IC}_{50} > 30 \mu\text{M}$).

Crystal Structure Determination

The intensity data were collected on a Nonius KappaCCD diffractometer, using graphite-monochromated $\text{Mo-K}\alpha$ radiation. Data were corrected for Lorentz and polarization effects; absorption was taken into account on a semi-empirical basis using multiple-scans.^[32]

The structure was solved by direct methods (SHELXS) and refined by full-matrix least squares techniques against Fo^2 (SHELXL-2018).^[33] The hydrogen atom bonded to hydroxy-group O1 were located by difference Fourier synthesis and refined isotropically. All other hydrogen atoms were included at calculated positions with fixed thermal parameters.

The analysis of the absolute structure using likelihood methods^[34] was performed using PLATON.^[35] The method calculated that the probability that the structure is inverted is smaller than 1×10^{-4} . The resulting value was $y = 0.0(2)$ indicating that the absolute structure has probably been determined correctly. The absolute structural parameter for the standard uncertainty using the Flack-parameter^[36] is $0.0(3)$.

XP (SIEMENS Analytical X-ray Instruments, Inc.) was used for structure representations.

Crystal Data for 3: C₁₅H₂₆O₂, Mr = 238.36 g mol⁻¹, colourless prism, size 0.125 × 0.110 × 0.090 mm³, orthorhombic, space group P 2₁ 2₁ 2₁, a = 8.3614(1), b = 11.1955(1), c = 14.8478(2) Å, V = 1389.90(3) Å³, T = -140 °C, Z = 4, ρ_{calcd.} = 1.139 g cm⁻³, μ (Mo-Kα) = 0.73 cm⁻¹, multi-scan, transmin: 0.6079, transmax: 0.7456, F(000) = 528, 12472 reflections in h(-10/10), k(-14/14), l(-19/19), measured in the range 2.744° ≤ Θ ≤ 27.487°, completeness Θ_{max} = 99.7%, 3189 independent reflections, R_{int} = 0.0311, 3084 reflections with F_o > 4σ(F_o), 162 parameters, 0 restraints, R_{1obs} = 0.0309, wR²_{obs} = 0.0846, R_{1all} = 0.0322, wR²_{all} = 0.0858, GOOF = 1.079, largest difference peak and hole: 0.247 / -0.144 e Å⁻³.

Data Deposition

Crystallographic data deposited at the Cambridge Crystallographic Data Centre under CCDC-1406841 for **3** contain the supplementary crystallographic data excluding structure factors; this data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Supplemental Material

Experimental details, details on x-ray crystallography, NMR data and spectra of compounds **1–3**. The raw NMR data ((FIDs) can be downloaded at https://files.dtu.dk/u/rg_qTOAP6ma8D-pN/NMR%20data%20files.zip

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Author Contribution Statement. Ling Ding and Helmar Görls performed the experiments, analyzed the data. Ling Ding, Christian Hertweck and Helmar Görls wrote the article.

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Table 1. NMR data for compounds **1–4**.

Position	1		4 ¹⁹	2		3	
	δ_{H} (<i>J</i> in Hz)	δ_{C} , type	δ_{C} , type	δ_{H} (<i>J</i> in Hz)	δ_{C} , type	δ_{H} (<i>J</i> in Hz)	δ_{C} , type
1	-	72.7, C	72.7, C	-	72.6, C	1.31 (overlapping)	49.1, CH
2	1.69 (overlapping), 1.58 (overlapping)	21.8, CH ₂	21.9, CH ₂	1.62 (overlapping)	21.8, CH ₂	1.51 (m), 1.19 (overlapping)	19.1, CH ₂
3	2.08 (overlapping)	26.6, CH ₂	26.6, CH ₂	2.12 (t, 8.6)	26.6, CH ₂	1.64 (m), 1.42 (m)	30.1, CH ₂
4	-	136.4, C	136.3, C	-	134.4, C	2.18 (m)	30.6, CH
5	5.63 (dq, <i>J</i> 5,7 = 1.6, <i>J</i> 5,6 = 6.1)	122.4, CH	122.5, CH	5.50 (dq, <i>J</i> 5,7 = 1.6, <i>J</i> 5,6 = 5.3)	122.1, CH	3.43 (dd, <i>J</i> 5,6 = 10.5, <i>J</i> 4,5 = 5.0)	81.5, CH
6	1.84 (dd, <i>J</i> 6,7 = 11.1, <i>J</i> 5,6 = 6.1)	47.9, CH	48.0, CH	1.79 (dd, <i>J</i> 6,7 = 11.3, <i>J</i> 5,6 = 5.3)	47.9, CH	1.18 (overlapping)	43.4, CH
7	1.35 (ddd, <i>J</i> 7,8 β = 12.4, <i>J</i> 6,7 = 11.1, <i>J</i> 7,8 α = 3.2)	54.9, CH	54.9, CH	1.25 (m)	47.8, CH	1.40 (dd, <i>J</i> 6,7 = 12.2, <i>J</i> 7,8 α = 3.8)	52.8, CH
8	1.74 (ddd, <i>J</i> 8 α , 8 β = 13.0, <i>J</i> 8 α , 9 α = 6.4, <i>J</i> 7, 8 α = 3.2),	28.8, CH ₂	28.9, CH ₂	1.61 (overlapping), 1.08 (overlapping)	26.9, CH ₂	1.62 (dd, <i>J</i> 8 α , 8 β = 6.7, <i>J</i> 7,8 α = 3.8), 1.20 (overlapping)	23.7, CH ₂

	1.04 (ddd, $J_{8\alpha, 8\beta}$ $=13.0$, J_7 , 8β $=12.4$, $J_{9\alpha}$, $8\beta = 3.2$)					)	
9	1.61 (overlapping), 1.16 (overlapping)	31.1, CH ₂	31.1, CH ₂	1.58 (overlapping), 1.06 (overlapping)	31.4, CH ₂	1.79 (dt, $J_{9\alpha, 9\beta} =$ 13.0 , $J_{8\alpha, 9\beta}$ $= 3.2$, $J_{8\beta, 9\beta}$ $= 3.2$); 1.50 (m)	43.3, CH ₂
10	1.60 (overlapping)	41.5, CH	41.5, CH	1.60 (overlapping)	41.8, CH	-	73.1, C
11	-	74.0, C	74.0, C	1.98 (m)	36.1, CH	-	81.6, C
12	1.13 (s)	30.2, CH ₃	30.2, CH ₃	3.42 (dd, $J_{11, 12A}$ $=10.3$, $J_{12A, 12B} =$ 10.3), 3.83 (dd, $J_{12A, 12B} =$ 10.3 , $J_{11, 12B}$ $= 5.0$)	65.0, CH ₂	1.02 (s)	24.6, CH ₃
13	1.18 (s)	24.7, CH ₃	24.8, CH ₃	0.94 (d, $J_{11, 13} = 6.4$)	15.8, CH ₃	1.23 (s)	28.9, CH ₃
14	0.94 (d, $J_{10, 14} = 6.7$)	15.2, CH ₃	15.2, CH ₃	0.95 (d, $J_{10, 14} = 6.6$)	15.1, CH ₃	1.17 (s)	21.7, CH ₃
15	1.70 (overlapping)	23.4, CH ₃	23.4, CH ₃	1.69 (s)	23.5, CH ₃	0.87 (d, J_4 , $15 = 7.0$)	10.6, CH ₃

Figures

Figure 1. Structures of representative cadinanes.

Figure 3. ORTEP drawing of crystal structure for **3**.

Scheme 1. Proposed biosynthetic pathway for isolated cadinane sesquiterpenes.

Graphical Abstract

Plant-like Cadinane Sesquiterpenes from an Actinobacterial Mangrove Endophyte

Ling Ding,* Helmar Görls, and Christian Hertweck

- Cadinanes are typical plant-derived sesquiterpenes with diverse bioactivity profiles.
- (+)-11-Hydroxy-epicubenol, (+)-12-hydroxy-epicubenol and 5,11-epoxy-10-cadinanol are reported here from a bacterial source.
- The discovery of oxygenated cadinanes from a bacterial endophyte may set the basis for the production of cadinanes by bacterial fermentation.