Pure Appl. Chem., Vol. 84, No. 9, pp. 1847–1855, 2012.
http://dx.doi.org/10.1351/PAC-CON-11-10-20

Bioactive cubitane diterpenoids from a Colombian gorgonian species of the genus *Eunicea**

Xiaomei Wei, Karinel Nieves, and Abimael D. Rodríguez‡

Department of Chemistry, University of Puerto Rico, P.O. Box 23346, U. P. R. Station, San Juan 00931-3346, Puerto Rico

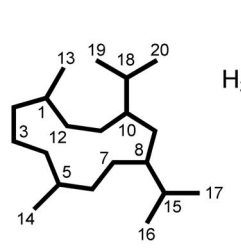
Abstract: Two new diterpenes having the rare cubitane skeleton were isolated from a southwestern Caribbean gorgonian coral of the genus *Eunicea*. The structures of the new metabolites, as well as those of three known cubitanes were elucidated by extensive spectroscopic analysis. One compound showed significant in vitro cytotoxic activity against a National Cancer Institute (NCI) panel of cancer cell lines, whereas the remaining metabolites were shown to exert mild antitubercular and antimalarial activities against the pathogenic microbes *Mycobacterium tuberculosis* and *Plasmodium falciparum*.

Keywords: anticancer activity; antimalarial activity; antitubercular activity; Caribbean gorgonian; *Eunicea* sp.; infectious deseases; isolation; marine chemistry; natural products; physicochemical characterization of cubitane diterpenes; structures.

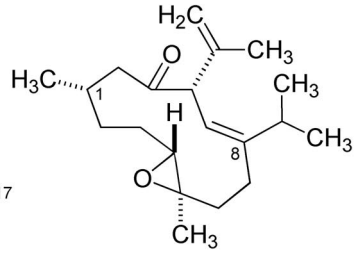
INTRODUCTION

Gorgonian corals of the genus *Eunicea* (family Plexauridae) have been shown to be a rich source of diterpenes possessing a variety of carbon skeletons [1]. In the Caribbean Sea, these sea whips are particularly abundant, and thus, it is not surprising that many chemical investigations of this taxonomically complex genus have been conducted since the late 1960s [2]. In two earlier reports, we described the structures of 15 new cembrane and dolabellane diterpenoids from an undescribed species of *Eunicea* collected in 2002 as part of an expedition to Old Providence Island, Colombia located off the Nicaraguan shelf in the southwestern Caribbean Sea [3,4]. In this paper, we report the structures of two new diterpenoids, **1** and **2**, which were isolated from the same gorgonian *Eunicea* sp. These monocarbocyclic metabolites are new examples of diterpenoids of the irregular cubitane ring system, which was first isolated in 1978 from the East African termite, *Cubitermes umbratus* [5]. In total, only eight cubitanes have been reported previously from one of the 15 varieties of *Eunicea* found in the Caribbean Sea, namely, *Eunicea laciniata* (Duchassaing and Michelotti) [6–8].

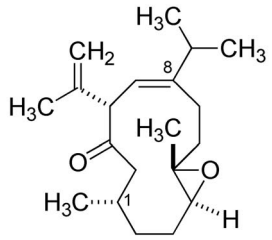
Pure Appl. Chem.* **84, 1837–1937 (2012). A collection of invited papers based on presentations on the Chemistry of Life theme at the 43rd IUPAC Congress, San Juan, Puerto Rico, 30 July–7 August 2011.
‡Corresponding author: Tel.: (787) 764 0000 Ext. 4799; Fax: (787) 756 8242; E-mail: abrodriguez@uprrp.edu



cubitane skeleton



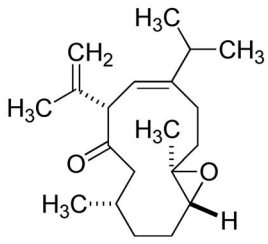
1



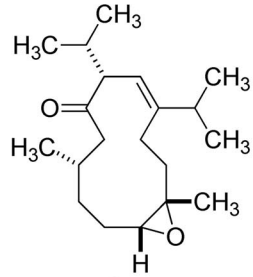
2

RESULTS AND DISCUSSION

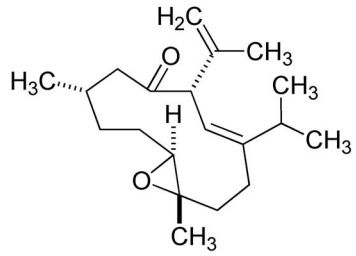
Collection of *Eunicea* sp. was made in deeper water (–26 m) along the reefs of Old Providence Island. Freshly collected animals were partially air dried, frozen, lyophilized, and later extracted with MeOH/CH_2Cl_2 (1:1). Calyculones H (**1**) and I (**2**), along with the previously known calyculones A–C (**3–5**) were isolated by rapid elution Si gel column chromatography (CC) of the hexane extract and purified after successive Si gel CC and high-performance liquid chromatography (HPLC) from approximately the same nonpolar fractions containing the recently reported dolabellane derivatives [4]. The five isomeric calyculones **1–5** were minor components of the hexane extract, each comprising slightly less than 0.04 % of the organic extract. Interestingly, the pseudopterane carbon skeleton is the only other known example of a 12-membered monocyclic diterpenoid ring system that contains two isopropyl groups, and both of these diterpenoid systems were considered as possibilities for the carbon skeleton of **1** and **2** [9,10].



3



4



5

Data from high-resolution electron impact mass spectroscopy (HREIMS) and ^{13}C NMR spectrometry (Table 1) established a molecular formula of $C_{20}H_{32}O_2$ for calyculone H (**1**). Evaluation of spectral information established the presence of a trisubstituted epoxide and a ketone, which accounted for the two oxygen atoms in the molecular formula. ^{13}C NMR signals at δ 62.5 (CH) and 59.9 (qC) along with ^{1}H NMR resonances at δ 2.73 (dd, 1H, J = 3.5, 9.8 Hz) and 1.30 (s, 3H) showed that the epoxide in **1** was methyl substituted. A ^{13}C NMR band at δ 211.3 (qC), coupled with an IR absorption at 1711 cm^{-1} and the lack of absorption in the UV spectrum further indicated that calyculone H possessed a nonconjugated ketone. The presence of an isopropenyl group in **1** was next established based upon signals in the ^{13}C NMR spectrum at δ 113.3 (CH_2) and 143.3 (qC), in conjunction with bands in the ^{1}H NMR spectrum at δ 4.88 and 4.77 (each, br s, 1H). These latter proton bands were allylically coupled to a broadened resonance at δ 1.72 (br s, 3H), assigned to a vinyl methyl. Two other resonances in the ^{13}C NMR spectrum of **1** at δ 146.4 (qC) and 122.6 (CH), as well as a signal in the ^{1}H NMR spectrum at δ 5.03 (br d, 1H, J = 9.3 Hz) showed the molecule to possess one other nonconjugated trisubstituted olefin. The olefin proton of this group was coupled to a highly deshielded proton at δ 4.29

(br d, 1H, J = 9.3 Hz) that was apparently bounded by two quaternary centers as it showed no further couplings.

Table 1 ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) assignments for compounds **1** and **2** in $CDCl_3$[a].

	Calyculone H (**1**)		Calyculone I (**2**)	
atom	δ_H, mult, intgr, J in Hz	δ_C (mult)	δ_H, mult, intgr, J in Hz	δ_C (mult)
1	2.17, m, 1H	27.7 (CH)	2.00, m, 1H	28.8 (CH)
2α	1.22, m, 1H	31.9 (CH_2)	1.18, m, 1H	30.3 (CH_2)
2β	1.35, m, 1H		1.43, m, 1H	
3α	2.01, m, 1H	25.0 (CH_2)	2.02, m, 1H	25.5 (CH_2)
3β	1.27, m, 1H		1.18, m, 1H	
4	2.73, dd, 1H, 3.5, 9.8	62.5 (CH)	2.55, m, 1H	63.2 (CH)
5		59.9 (qC)		60.8 (qC)
6α	2.07, m, 1H	37.1 (CH_2)[b]	2.12, m, 1H	36.0 (CH_2)
6β	1.43, m, 1H		1.37, m, 1H	
7α	2.08, m, 1H	28.0 (CH_2)[b]	2.05, m, 1H	24.8 (CH_2)
7β	2.28, m, 1H		2.48, m, 1H	
8		146.4 (qC)		146.8 (qC)
9	5.03, br d, 1H, 9.3	122.6 (CH)	5.54, d, 1H, 10.3	120.3 (CH)
10	4.29, br d, 1H, 9.3	57.1 (CH)	4.36, d, 1H, 10.3	54.6 (CH)
11		211.3 (qC)		210.2 (qC)
12α	2.16, m, 1H	55.0 (CH_2)	2.07, m, 1H	52.4 (CH_2)
12β	2.55, d, 1H, 7.9		2.63, dd, 1H, 3.9, 13.2	
13	1.05, d, 3H, 5.8	20.0 (CH_3)	1.00, d, 3H, 6.3	23.5 (CH_3)
14	1.30, s, 3H	16.6 (CH_3)	1.38, s, 3H	17.5 (CH_3)
15	2.75, m, 1H	29.7 (CH)	2.24, m, 1H	31.1 (CH)
16	0.97, d, 3H, 7.0	21.6 (CH_3)	0.97, d, 3H, 6.9	20.5 (CH_3)
17	1.06, d, 3H, 7.0	21.4 (CH_3)	1.07, d, 3H, 6.9	21.5 (CH_3)
18		143.3 (qC)		143.3 (qC)
19	1.72, br s, 3H	21.5 (CH_3)	1.69, br s, 3H	20.3 (CH_3)
20α	4.88, br s, 1H	113.3 (CH_2)	4.82, br s, 1H	112.7 (CH_2)
20β	4.77, br s, 1H		4.79, br s, 1H	

[a]Proton and carbon chemical shift values are in ppm relative to TMS and $CDCl_3$ signal (77.0 ppm), respectively. Spectra were recorded at 25 °C. Proton assignments were aided by ^{1}H–^{1}H COSY, HMQC, HMBC, and NOESY experiments.

[b]Low-intensity broad resonance line.

Further analysis of the ^{1}H NMR spectrum of calyculone H showed the presence of three doublet methyls at δ 0.97 (d, 3H, J = 7.0 Hz), 1.05 (d, 3H, J = 5.8 Hz), and 1.06 (d, 3H, J = 7.0 Hz). The ^{1}H–^{1}H COSY spectrum showed that the one-proton multiplet at δ 2.75 was coupled to two of the doublet methyls (δ 0.97 and 1.06), thereby establishing their assignment in an isopropyl group. The isopropyl methine at δ 2.75 was found to be further coupled only to the olefinic proton at δ 5.03 by a small (<1 Hz) allylic coupling constant, thus establishing the isopropyl group as a substituent of the trisubstituted olefinic bond. From these data, four of the five degrees of unsaturation in the molecular formula of **1** could be accounted for, demonstrating that calyculone H was monocarbocyclic.

In the ^{1}H–^{1}H COSY spectrum, it was possible to identify four different structural units, which were assembled with the assistance of an heteronuclear multiple-bond correlation (HMBC) experiment (Fig. 1). Key HMBC correlations of H-10 (δ_H 4.29) to C-11 (δ_C 211.3), C-12 (δ_C 55.0), C-18 (δ_C 143.3), C-19 (δ_C 21.5), and C-20 (δ_C 113.3); H_3-14 (δ_H 1.30) to C-4 (δ_C 62.5), C-5 (δ_C 59.9), and C-6

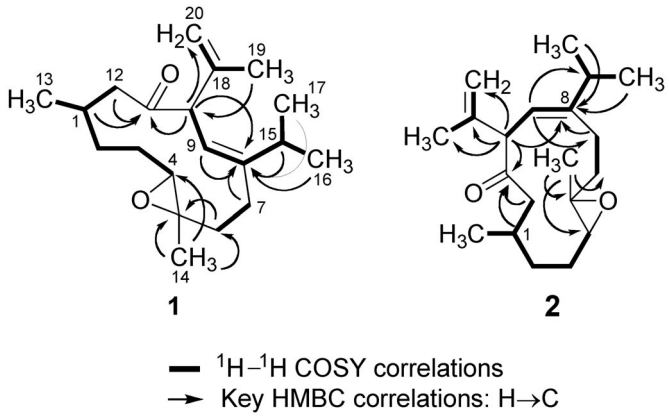
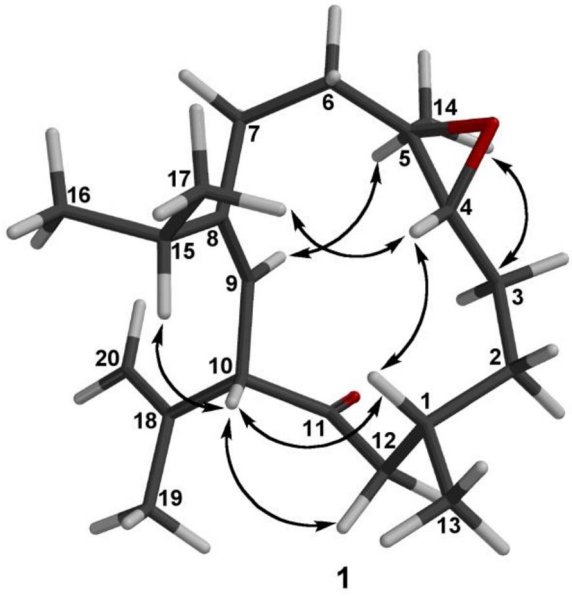


Fig. 1 Partial structures for **1** and **2** based on key ^{1}H–^{1}H COSY and HMBC correlations.

(δ_C 37.1); and H_2-7 (δ_H 2.28 and 2.08) to C-5 (δ_C 59.9), C-8 (δ_C 146.4), C-9 (δ_C 122.6), and C-15 (δ_C 29.7) permitted connection of the 12-membered carbon skeleton. On the basis of the above analysis, the planar structure of **1** was established unambiguously. Furthermore, comparison of ^{1}H and ^{13}C NMR data of **1** (Table 1) with those of isomer calyculone C (**5**) showed that the structures of both compounds are very similar [6]. More critically, analysis of the ^{13}C NMR data also revealed, in comparison to compound **5**, that the resonances ascribed to the C-4,5 epoxide and associated methyl carbon C-14 were somewhat shifted. Calyculones H (**1**) and C (**5**) were, therefore, concluded to be geometric isomers about the trisubstituted epoxide functionalities.

The relative structure of **1** was elucidated by the analysis of nuclear Overhauser effect (NOE) correlations, as shown in Figure 2. It was found that the C-15 isopropyl methine proton and the proton at C-10 were within NOE proximity. Therefore, in contrast to calyculones A (**1**) and B (**2**), the stereochemistry of the trisubstituted olefin was defined as *Z* in compound **1** [6]. Since H-10 also showed NOE interactions with H-1 and one of the methylene protons at C-12 (δ 2.55), assuming a β-orientation of H-10, the latter protons should also be positioned on the β-face. Since no cross-peaks were observed between the epoxide proton (H-4) and its associated methyl resonance (H_3-14), a *trans* geometry was assigned to this group. On the other hand, the fact that H-4 exhibited NOE correlations with both H-1 and one of the isopropyl methyls (δ 1.06), reflected its β-orientation and hence the *R** configuration at C-4. Furthermore, H_3-14 exhibited NOE correlations with H-9 (δ 5.03) and one of the methylene protons at C-3 (δ 2.01), revealing the α-orientation of H_3-14, and hence the *R** configuration of C-5. Overall, these results established the spatial arrangement of the proton at C-10, the epoxide proton H-4, the isopropyl group, and the proton at C-1 on the β-face of the molecule. These studies established that calyculone H (**1**) only differed from calyculone C (**5**) in the geometry of the epoxide (also *trans*, but opposite). From these results, the structure of calyculone H (**1**) was assigned as 4(*R**),5(*R**)-epoxy-11-keto-1(*S**),10(*S**)-cubita-8(*Z*),18(20)-diene.

Calyculone I (**2**) also analyzed for $C_{20}H_{32}O_2$ by HREIMS and showed IR and ^{13}C NMR bands that suggested the presence of carbonyl and epoxide functionalities similar to those found in calyculone H (**1**). Moreover, the NMR features of calyculone I (Table 1) also compared favorably to those of calyculone A (**3**), showing the presence in each isomer of similar functional groups and substituents [6]. The proton correlations observed in the ^{1}H–^{1}H COSY showed the presence of four partial structures, as illustrated in Fig. 1. After assignment of all the direct ^{13}C–^{1}H correlations were made on an heteronuclear multiple-quantum coherence (HMQC) analysis, the HMBC was measured and analyzed to obtain the gross structure of **2**. Correlations of H-10 to C-11, C-18, C-19, and C-20, from H_3-14 to C-4, C-5, and C-6, and from H_2-7 to C-8, C-9, and C-15 connected all of the partial structures deduced from



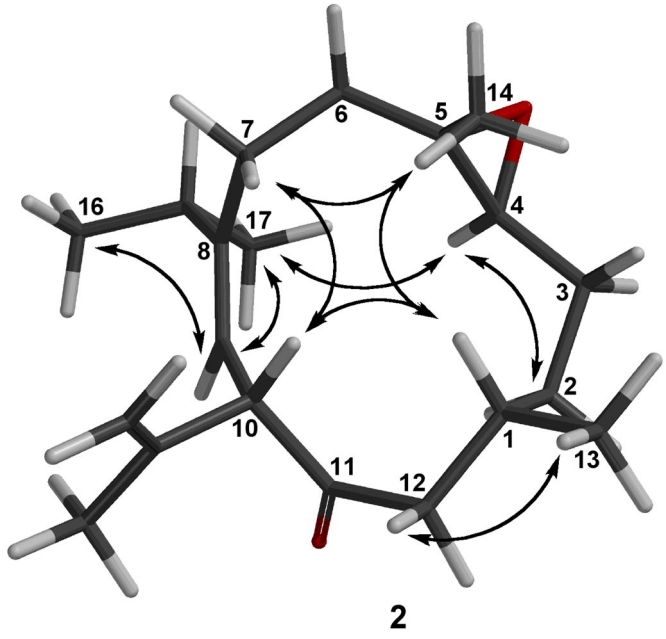
1

Fig. 2 Key NOESY correlations for calyculone H (**1**).

the ^{1}H–^{1}H COSY spectrum. On the basis of the above data, we concluded that calyculone I (**2**) possesses the same planar structure as that of calyculone H (**1**), which indicated that these compounds must be geometric isomers about the trisubstituted olefin and/or the epoxide functionalities.

From the NOESY spectrum (Fig. 3), it was found that H-9 (δ 5.54, d, J = 10.3 Hz) showed strong NOE interactions with the isopropyl methyls H_3-16 (δ 0.97, d, J = 6.9 Hz) and H_3-17 (δ 1.07, d, J = 6.9 Hz) indicating the *E* stereochemistry of the trisubstituted olefin. Meanwhile, the NOESY spectrum showed no cross-peaks between epoxide proton H-4 and its associated methyl resonance H_3-14, suggesting a *trans* relationship. Further analysis of the NOESY spectrum revealed that H-10 (δ 4.36, d, J = 10.3 Hz) gave an NOE interaction with both H-1 (δ 2.00, m) and H-7β (δ 2.48, m), but not with H-15. In addition, H-1 showed NOE cross-peaks with H_3-14, which in turn strongly interacted with H-7β, revealing the β-orientation of H-1, H-10, and H_3-14. Furthermore, the epoxide proton H-4 (δ 2.55, m), one of the isopropyl methyls (δ 1.07), and one of the methylene protons at C-2 (δ 1.18, m) were found to be within NOE proximity on the opposite α-face of the molecule. Therefore, **2** was found to be 4(*S**),5(*S**)-epoxy-11-keto-1(*S**),10(*S**)-cubita-8(*E*),18(20)-diene, the C-4,5 bis-epimer of calyculone A (**3**).

Insofar as no attempts were made during previous investigations to screen *Eunicea* sp. cubitanes for chemotherapeutic activity, compounds **1**, **3**, **4**, and **5** were tested for in vitro antituberculosis (*Mycobacterium tuberculosis*), antimalarial (*Plasmodium falciparum*), and human cancer cell cytotoxic activities. Thus, at a concentration of 6.25 μg/mL these compounds were found to be marginally active against *M. tuberculosis* as their percentages of inhibition only ranged between 23–43 %. On the other hand, when tested for their inhibitory activity toward the growth of *Plasmodium falciparum*, they showed mild antiplasmodial activity with IC_{50} values of 11, 5, 8, and 10 μg/mL, respectively. One of the present isolates, calyculone A (**3**), was assayed in the National Cancer Institute (NCI) in vitro antitumor screen consisting of 60 human tumor cell lines selectively displaying strong cytotoxicity against SR leukemia, melanoma UACC-62, and RXF 393 renal cancer. Thus, the averaged GI_{50} values (the concentration of compound required for 50 % growth inhibition) were 1.94×10^{-7}, 1.88×10^{-8}, and 2.94×10^{-7} μM, respectively.



2

Fig. 3 Key NOESY correlations for calyculone I (**2**).

CONCLUSION

The extraction and chemical evaluation of *Eunicea* gorgonian species for novel natural products continue to be an endeavor of increasing importance owing to the potential for the discovery of secondary metabolites with optimum medicinal properties. However, the intrinsic difficulties associated with the accurate taxonomic identification of *Eunicea* species, and in particular the complex nature of marine natural products, have meant that accessing new structural variants has often been limited [11]. Interestingly, while the structures of compounds **1** and **2** possess similar functionalities to those of previously known *Eunicea* cubitanes, diterpenes based on this irregular carbon skeleton continue to be among the most inconspicuous of all the natural products reported thus far [1,12]. These analogs have shown a spectrum of results in initial cell culture and antimicrobial evaluations. Further elaboration of this ring functionality [13], including determination of its absolute structure, is currently in progress in our laboratory.

EXPERIMENTAL

General experimental procedures

Optical rotations were recorded with a Rudolph Autopol IV polarimeter. The IR analyses were performed with a Nicolet Magna IR 750FT-IR spectrometer. 1D and 2D NMR data were recorded on a Bruker DPX-300 spectrometer. ^{1}H NMR chemical shifts were recorded at 25 °C with respect to TMS and ^{13}C NMR chemical shifts were recorded in ppm relative to $CDCl_3$ (77.0 ppm). Mass spectrometric measurements were generated at the Mass Spectrometry Laboratory of the University of Illinois at Urbana–Champaign. CC was performed using Si gel (35–75 mesh) or bonded C18 Si gel (35–75 mesh), and thin-layer chromatography (TLC) analysis was carried out using glass precoated Si gel plates and the spots were visualized using a UV lamp at λ = 254 nm or by exposure to I_2 vapor. HPLC was performed using either an Ultrasphere polar-bonded Cyano semi-preparative column (5 µ, 10 mm × 25 cm) or an Ultrasphere ODS reversed-phase Si gel semi-preparative column (5 µ, 10 mm × 25 cm). All HPLC

separations were monitored simultaneously with a refractive index detector and a UV detector set at λ = 220 nm using a flow rate = 2 mL/min with isocratic elution of the mobile phase. All solvents were distilled from glass prior to use. Lowest energy conformers for the new compounds were searched using MMFF force field implemented in the McSpartan '04 Program (Wavefunction, Inc.). We have trivially named compounds **1** and **2** as calyculones H and I, respectively, in keeping with previous works by Fenical and co-workers [6,7]. The percentage yield of each compound is based on the weight of the crude hexane extract.

Animal material

A detailed taxonomical description of the biological specimens used in this investigation has been provided previously [3].

Collection, extraction, and isolation

Medium to large colonies (0.5–1.3 m) of the gorgonian coral *Eunicea* sp. (undescribed species; order: Gorgonacea; family: Gorgoniidae; phylum: Cnidaria) were collected by SCUBA at depths of 75–85 ft from the coral reefs off Old Providence Island (March, 2002), Colombia located off the Nicaraguan shelf in the southwestern Caribbean Sea. A voucher specimen (No. *Eunicea* sp. 2) is on deposit at the Chemistry Department of the University of Puerto Rico. The gorgonian specimens were partially air-dried, freeze-dried, and then kept frozen prior to extraction. The dried animal (1.7 kg) was blended with a mixture of 1:1 MeOH/CH_2Cl_2 (5 × 4 L) and, after filtration, the combined extracts were concentrated in vacuo to afford a gummy green residue (74.4 g). The crude extract was suspended in water (1L) and extracted successively with hexane (4 × 2 L), CH_2Cl_2 (4 × 2 L), and EtOAc (3 × 2 L). The hexane extract (45.7 g) was chromatographed on a large Si gel column by stepwise elution with 100 % hexane, hexane/EtOAc mixtures (100–0 %), and then 100 % MeOH. Fractions were pooled based on their TLC and NMR profile to yield 15 primary fractions, denoted I–XV. After fraction IV (670 mg) was fractionated by Si gel (30 g) CC with stepwise elution using hexane–acetone mixtures (1–10 %), the least polar fraction to elute (40 mg) was purified successively by reversed-phase HPLC (ODS column with 4:1 MeOH/H_2O), normal-phase HPLC (Cyano column with 9:1 hexane/2-propanol), and Si gel CC with hexane–EtOAc (30:1) to yield calyculone I (**2**) (8.0 mg, 0.02 %). On the other hand, the more polar fraction eluting out (59 mg) was purified by Si gel (2.0 g) CC with 50:1 hexane–EtOAc yielding known calyculone C (**5**, 8.5 mg, 0.02 %) [6]. Fraction V (773 mg) was fractionated by Si gel (30 g) CC with stepwise elution using hexane–acetone mixtures (0–100 %) to yield nine secondary fractions. The third subfraction (230 mg) was purified twice by Si gel (10 g) CC with hexane–EtOAc (80:1) to yield known calyculones A (**3**, 14 mg, 0.03 %) and B (**4**, 16 mg, 0.035 %) [6]. Fraction VII (420 mg) was fractionated by size-exclusion CC on a Bio-Beads SX-3 column (90 g, toluene), and the penultimate fraction eluted (155 mg) was purified successively by Si gel CC [18 g, elution with hexane–acetone (80:1)] and reversed-phase ODS Si gel CC [10 g, elution with MeOH–H_2O (65:35)] yielding calyculone H (**1**, 13 mg, 0.03 %).

Calyculone H [4(*R**),5(*R**)-e poxy-11-keto-1(*S**),10(*S**)-cubita-8(*Z*),18(20)-diene] (1)

Colorless oil, $[\alpha]^{20}_D$ –69.0 (*c* 1.3, $CHCl_3$); IR (film) ν_{max} 2964, 2932, 2870, 1711, 1624, 1455, 1379, 1240, 1070, 892 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) (see Table 1); EIMS *m/z* $[M]^+$ 304 (3), 302 (9), 289 (2), 261 (6), 136 (72), 121 (100), 107 (31), 93 (73); HREIMS *m/z* $[M]^+$ 304.2394 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Calyculone I [4(*S**),5(*S**)-epoxy-11-keto-1(*S**),10(*S**)-cubita-8(*E*),18(20)-diene] (2)

Colorless oil, $[\alpha]^{20}_{D}$ +52.1 (*c* 1.0, $CHCl_3$); IR (film) ν_{max} 3079, 2960, 2935, 2874, 1708, 1460, 1381, 1261, 1094, 1072 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) (see Table 1); EIMS *m/z* $[M]^+$ 304 (2), 289 (2), 261 (6), 136 (85), 121 (100), 107 (23), 93 (61); HREIMS *m/z* $[M]^+$ 304.2398 (calcd for $C_{20}H_{32}O_2$, 304.2402).

Biological screening assays

Anticancer activity screening by the Developmental Therapeutics Program (DTP) of the NCI is conducted following this general protocol: most of the compounds screened have no antiproliferative activity (up to 85 %). In order to avoid screening inactive compounds across all the cell lines, a prescreen is done using three highly sensitive cell lines (breast MCF-7, lung NCI-H640, CNS SF-268). Antiproliferative activity must be seen in these cell lines in order to continue to the 60 cell line panel. The 60 different human tumor lines are incubated with five different doses of compound, and a sulforhodamine blue (SRB) assay is performed after 48 h to determine cytotoxicity. From the 5-point curve, the following concentrations are extrapolated: GI_{50} (inhibits growth by 50 %), TGI (totally inhibits growth), LC_{50} (kills 50 % of cells). For the specific screening methods from the DTP website, visit: <http://www.dtp.nci.nih.gov/branches/btb/ivclsp.html>. Compounds shown to have anticancer activity in cell lines within the NCI 60 panel may then move on to animal trials and if successful, may eventually move on to be tested in clinical trials. Experimental details for our primary in vitro antimicrobial assays against *Mycobacterium tuberculosis* and *Plasmodium falciparum* have been previously described [14,15].

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health-SC1 Program (Grant 1SC1GM086271-01A1) awarded to A. D. Rodríguez. We thank Juan A. Sánchez for assistance during the collection and identification of the gorgonian coral and Carlos Jiménez-Romero for efforts to re-isolate cubitanes **1–5**. Mass spectrometry determinations were provided by the Mass Spectrometry Laboratory of the University of Illinois at Urbana–Champaign. We gratefully acknowledge the assistance of Eduardo Ortega-Barria and José González from the Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT) in Panama for the antimalarial bioassays. The NCI and the TAACF provided in vitro cytotoxicity and antituberculosis activity data, respectively.

REFERENCES

1. F. Berrue, R. G. Kerr. *Nat. Prod. Rep*. **26**, 681 (2009).
2. A. D. Rodríguez. *Tetrahedron* **51**, 4571 (1995).
3. X. Wei, A. D. Rodríguez, P. Baran, R. G. Raptis, J. A. Sánchez, E. Ortega-Barria, J. González. *Tetrahedron* **60**, 11813 (2004).
4. X. Wei, A. D. Rodríguez, P. Baran, R. G. Raptis. *J. Nat. Prod*. **73**, 925 (2010).
5. G. D. Prestwich, D. F. Weimer, J. Meinwald, J. Clardy. *J. Am. Chem. Soc*. **100**, 2560 (1978).
6. S. A. Look, W. Fenical, Z. Qi-tai, J. Clardy. *J. Org. Chem*. **49**, 1417 (1984).
7. J. Shin, W. Fenical. *J. Org. Chem*. **56**, 1227 (1991).
8. K. I. Marville, W. F. Reynolds, R. L. Sealy, W. F. Tinto. *Heterocycles* **63**, 107 (2004).
9. M. M. Bandurraga, W. Fenical, S. F. Donovan, J. Clardy. *J. Am. Chem. Soc*. **104**, 6463 (1982).
10. J. Marrero, C. A. Ospina, A. D. Rodríguez, P. Baran, H. Zhao, S. G. Franzblau, E. Ortega-Barria. *Tetrahedron* **62**, 6998 (2006).
11. J. A. Sánchez. *Zool. J. Linn. Soc*. **157**, 237 (2009).

12. J. W. Blunt, B. R. Copp, M. H. G. Munro, P. T. Northcote, M. R. Prinsep. *Nat. Prod. Rep*. **28**, 196 (2011) and previous reports in this series.
13. E. Schöttner, M. Wiechoczek, P. G. Jones, T. Lindel. *Org. Lett*. **12**, 784 (2010).
14. L. A. Collins, S. G. Franzblau. *Antimicrob. Agents Chemother*. **41**, 1004 (1997).
15. Y. Corbett, L. Herrera, J. González, L. Cubilla, T. Capson, P. D. Colley, T. A. Kursar, L. I. Romero, E. Ortega-Barria. *J. Trop. Med. Hyg*. **70**, 119 (2004).

