JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

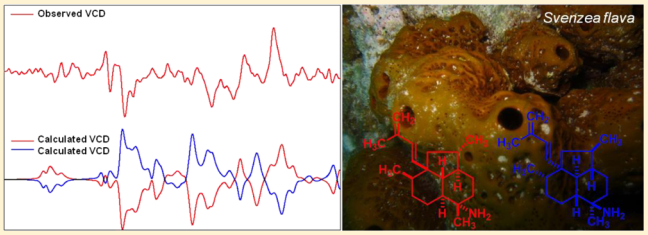
Two Rare-Class Tricyclic Diterpenes with Antitubercular Activity from the Caribbean Sponge *Svenzea flava*. Application of Vibrational Circular Dichroism Spectroscopy for Determining Absolute Configuration

Edward Avilés, Abimael D. Rodríguez,* and Jan Vicente

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

Supporting Information

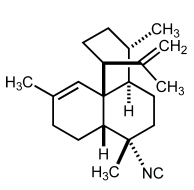
ABSTRACT: Two new natural products, **3** and **4**, and their predecessor 7-isocyanoisoneoamphilecta-1(14),15-diene (**2**), of the rare isoneoamphilectane class of marine diterpenes, along with the known amphilectane diterpenes **6**–**8**, were isolated from the *n*-hexane extract of the marine sponge *Svenzea flava* collected at Great Inagua Island, Bahamas. The molecular structures of compounds **3** and **4** were established by spectroscopic (1D/2D NMR, IR, UV, HRMS) methods and confirmed by a series of chemical correlation studies. In a first ever case study of the assignment of the absolute configuration of a molecule based on the isoneoamphilectane carbon skeleton, the absolute configuration of compound **5** was established as 3*S*,4*R*,7*S*,8*S*,11*R*,12*S*,13*R* by application of vibrational circular dichroism (VCD). In vitro anti-TB screenings revealed that metabolites **2**–**4** and, in particular, semisynthetic analogue **5**, are strong growth inhibitors of *Mycobacterium tuberculosis* H_{37}Rv.



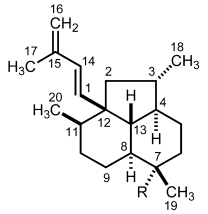
INTRODUCTION

Diterpenes based on the amphilectane ring skeleton and its variants (Scheme 1), possessing isocyanide, isocyanate, isothiocyanate, and formamide functionalities, have been isolated from marine sponges belonging to the genera *Hymeniacidon*,[1] *Cribochalina*,[2] *Stylissa*,[3] *Cymbastela*,[4] *Amphimedon* (*Adocia*),[5] *Halichondria*,[6] *Axinella*,[7] and *Pseudoaxinella*.[8] Among these polycyclic compounds, those that are based on the neoamphilectane and isoneoamphilectane scaffolds are considered exceptionally rare, and to this date, isocyanides **1** and **2** remain as the first and only known representatives of these families of marine-derived diterpenes, respectively.[9−11] Specifically, tricyclic compound **1** was isolated in 1992 by Higa and co-workers from a Japanese sponge of the family Adocidae,[9] whereas **2**, formally a 2(1→12)-abeoamphilectane, was isolated in 1996 by König et al. from the tropical marine sponge *Cymbastela hooperi*.[10] Often, amphilectane-based diterpenes known to contain isocyanide and related functionalities are endowed with potent in vitro antimalarial, antialgal, antitubercular, antibacterial, antiphotosynthetic, anti-inflammatory, antiproliferative, and antifouling activity.[8,10,12] Several investigations into the structure−activity relationships of these metabolites clearly suggest a connection between such nitrogen-containing functionalities and the observed patterns of pharmacological activity.[12]

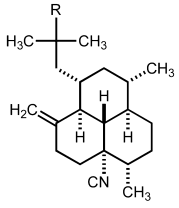
As part of our ongoing quest for novel biologically active natural products from marine sponges of the Caribbean region, we report herein on the extraction, isolation, and structure elucidation of two new isoneoamphilectane-based diterpenes,



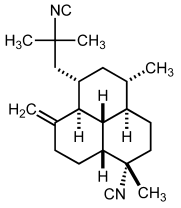
1



2 R = NC
3 R = $NHCH_3$
4 R = NHCHO
5 R = NH_2



6 R = NC
7 R = NHCHO



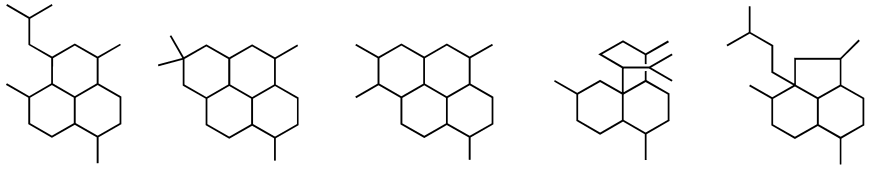
8

7-methylaminoisoneoamphilecta-1(14),15-diene (**3**) and 7-formamidoisoneoamphilecta-1(14),15-diene (**4**), from the *n*-hexane extract of the Bahamian sponge *Svenzea flava* (Lehnert and van Soest, 1999) (Phylum Porifera; Class Demospongiae; Order Halichondrida; Family Dictyonellidae).[13,14] Their structures, including relative configuration, were elucidated on

Received: August 20, 2013
Published: October 18, 2013

dx.doi.org/10.1021/jo401846m | *J. Org. Chem.* 2013, 78, 11294−11301

Scheme 1. Carbon Backbones for a Series of Spongian Amphilectane-Class Diterpenes and Their Trivial/Semisystematic Names[a]



[a]The names shown are consistent with the reports of Wright and Lang-Unnasch[4b] and König et al.[10] The numbers inside parentheses refer to the current number of sponge-derived analogues isolated per class of compound.

Table 1. ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{1}H−^{1}H COSY, NOESY, and HMBC Spectroscopic Data for 7-Methylaminoisoneoamphilecta-1(14),15-diene (3)[a]

position	δ_H, mult (J in Hz)	δ_C, type[b]	COSY	NOESY	HMBC[c]
1	5.68, d (16.1)	138.0, CH	H14	H2β, H10β, H17, H19, H20	H2$\alpha\beta$, H11, H13, H14
2α	1.00, br m	49.0, CH_2	H2β, H3	H2β	H1, H3, H11, H13, H18
2β	2.12, dd (12.7, 5.5)		H2α, H3	H1, H2α, H3, H14	
3	1.42, br m	37.3, CH	H2$\alpha\beta$, H4, H18	H2β, H14	H2$\alpha\beta$, H4, H5$\alpha\beta$, H13, H18
4	0.85, br m	54.9, CH	H3, H5α, H13	H5α, H8	H2, H3, H5$\alpha\beta$, H6$\alpha\beta$
					H8, H13, H18
5α	1.80, br m	27.3, CH_2		H4, H6α, H18, H21	H3, H4, H6$\alpha\beta$
5β	1.11, br m		H4, H6α	H5α	
6α	1.41, br m	37.9, CH_2		H5α, H6β	H4, H5$\alpha\beta$, H8, H19
6β	1.61, br m		H5β	H6α, H19	
7		56.1, C			H5$\alpha\beta$, H6$\alpha\beta$, H8,
					H9, H13, H19, H21
8	1.46, br m	44.7, CH	H9β	H4, H21	H4, H6$\alpha\beta$, H9$\alpha\beta$, H10$\alpha\beta$, H13
9α	0.96, br m	19.4, CH_2		H9β, H10α	H8, H11, H10$\alpha\beta$, H13
9β	1.58, br m		H8, H10β	H9α, H10β, H19	
10α	1.58, br m	28.5, CH_2		H9α, H10β	H8, H9$\alpha\beta$, H11, H20
10β	1.25, br m		H9β, H11	H1, H9β, H10α, H20	
11	1.57, br m	35.7, CH	H10β, H20		H1, H2$\alpha\beta$, H9$\alpha\beta$,
					H10$\alpha\beta$, H13, H20
12		46.1, C			H1, H2$\alpha\beta$, H4, H8, H10$\alpha\beta$, H11, H13, H14, H20
13	1.31, br m	54.2, CH	H4	H14, H19	H1, H2$\alpha\beta$, H3, H4, H5$\alpha\beta$, H8, H9$\alpha\beta$, H11
14	6.11, d (16.1)	129.4, CH	H1	H2α, H3, H13	H1, H16, H17
15		142.5, C			H1, H14, H16, H17
16	4.89, s	114.1, CH_2			H14, H17
17	1.84, s	18.9, CH_3		H1	H14, H16
18	0.92, d (6.4)	17.0, CH_3	H3	H5α	H2$\alpha\beta$, H3, H4
19	0.97, s	19.0, CH_3		H1, H6β, H9β, H13, H21	H6$\alpha\beta$, H8
20	0.76, d (6.4)	15.9, CH_3	H11	H1, H10β	H11, H10$\alpha\beta$
21	2.33, s	28.0, CH_3		H5α, H8, H19	

[a]Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in ppm 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 types were obtained from a DEPTQ experiment. [c]Protons correlated to carbon resonances in the ^{13}C column. Parameters were optimized for $^{2,3}J_{CH}$ = 6 and 8 Hz.

the basis of 1D and 2D NMR in conjunction with IR, UV, and HRMS spectroscopic evidence. Furthermore, the absolute configuration of **2**−**4** was determined indirectly via density functional theory (DFT) and calculated vibrational circular dichroism (VCD) techniques.[15] Briefly, we calculated the VCD and IR spectra for both enantiomers of semisynthetic derivative **5**, which were subsequently compared to its experimental VCD spectrum thus establishing the absolute configuration about its stereogenic centers. All isolates, but especially analogue **5**, demonstrated strong in vitro activity against the tuberculosis causative pathogen *Mycobacterium tuberculosis* H_{37}Rv.

RESULTS AND DISCUSSION

Isolation and Structure Elucidation of Diterpenes 3 and 4. A small specimen of *S. flava* (190 g of lyophilized sponge material) was extracted repeatedly with a 1:1 mixture of $CHCl_3$−MeOH, and after filtration and concentration of the combined extracts, the orange gum isolated was suspended in H_2O and partitioned against *n*-hexane. In vitro antituberculosis screening of the dry *n*-hexane solubles revealed significant inhibitory activity against *M. tuberculosis* H_{37}Rv (MIC = 15.1 μg/mL). Bioassay-guided fractionation of the extract using vacuum liquid chromatography (VLC) over silica gel in tandem

dx.doi.org/10.1021/jo401846m | J. Org. Chem. 2013, 78, 11294−11301

with NMR ($^1H/^{13}C$), IR, and TLC analyses of the active fractions led subsequently to the isolation of two new metabolites, 7-methylaminoisoneoamphilecta-1(14),15-diene (**3**) and 7-formamidoisoneoamphilecta-1(14),15-diene (**4**), along with the following known compounds: 7-isocyanoisoneoamphilecta-1(14),15-diene (**2**),[10] (−)-8,15-diisocyano-11(20)-amphilectene (**6**),[1a] 8-isocyano-11(20)-ene-15-amphilectaformamide (**7**),[1a] and 7,15-diisocyano-11(20)-amphilectene (**8**).[2,16] All of the known isolates were characterized unambiguously by spectroscopic analysis, including ESI-MS, UV, IR, $[\alpha]_D$, and NMR spectra, and by comparisons with data from previously published reports.

The ESI-MS (positive ions) of 7-methylaminoisoneoamphilecta-1(14),15-diene (**3**), isolated as a colorless oil, showed a pseudomolecular ion peak at m/z 302 $[M + H]^+$. Accurate mass measurement of this peak assigned the molecular formula $C_{21}H_{35}N$ to **3**. The IR spectrum revealed bands that were consistent with the presence of N−H (3404 cm^{-1}) and alkene (3078, 1639, 970, and 881 cm^{-1}) functionalities. The presence of four peaks in the ^{13}C NMR spectrum ascribable to sp^2-hybridized carbons at δ_C 142.5 (C, C-15), 138.0 (CH, C-1), 129.4 (CH, C-14), and 114.1 (CH_2, C-16) established the presence of two carbon—carbon double bonds that had the aspects of being conjugated [UV(hexane), λ_{max} 234 nm (ε 16000)]. The ^1H and ^{13}C NMR spectra of **3** ($CDCl_3$, Table 1) were analyzed with the help of a 2D NMR HSQC experiment, suggesting the presence of four methyls at δ_H 1.84 (s), 0.97 (s), 0.92 (d, J = 6.4 Hz), and 0.76 (d, J = 6.4 Hz), an additional methyl singlet at δ_H 2.33, six methylenes (one sp^2 and five sp^3), seven methines (two sp^2 and five sp^3), two quaternary carbons (one sp^2 and one sp^3), and one unprotonated sp^3 carbon atom bearing nitrogen. Thus, in order to account for the five unsaturation degrees implied by the molecular formula, **3** must be tricyclic. After all proton and carbon resonances had been associated from the results of $^1H-^{13}C$ shift correlated 2D NMR measurements (HSQC, J = 150 MHz), it was possible to deduce the planar structure of **3** from data subtracted from its $^1H-^1H$ DQF-COSY and HMBC (J = 6.0 and 8.0 Hz) spectra. Critically, inspection of the $^1H-^1H$ COSY spectrum allowed us to deduce the two spin systems evidenced with bold linkages in Figure 1, while analysis of the HMBC spectrum provided

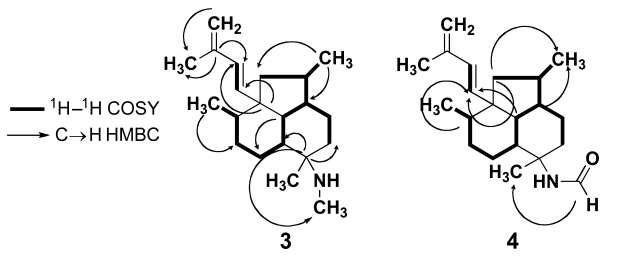


Figure 1. Spin systems deduced through the $^1H-^1H$ DQF-COSY spectra and key $^{2,3}J$ C→H correlations exhibited by the HMBC spectra of compounds **3** and **4**.

information to join the above moieties, thus building up the planar structure of compound **3** (Table 1). In particular, $^{2,3}J$ HMBC correlations of the methyl protons appended at C-3, C-7, and C-11 indicated the presence of a perhydroacenaphthene ring that, in turn, was connected to the C_5 acyclic tail thanks in no small part to the simultaneous correlations of C-12 (δ_C 46.1, C) with H-1, H-2$\alpha\beta$, H-11, H-13, and H_3-20.[17] After its ^1H and ^{13}C NMR data had been recorded and assigned (Table 1), it dawned upon us that **3** was essentially identical to prototype **2** with the exception of the substituent at C-7. On the basis of IR, ESI-MS, and NMR (^1H and ^{13}C) spectroscopic evidence, this substituent clearly had to be a methylamino function. The proposed structure elucidation of **3** was subsequently confirmed by analysis of the HSQC-TOCSY $^{13}C-^1H$ shift correlated spectrum to furnish its structure as a new isoneoamphilectane congener, namely, 7-methylaminoisoneoamphilecta-1(14),15-diene.

Stereochemically, **3** was proposed to be identical to **2** on the basis of the almost identical ^{13}C NMR data and optical rotation comparisons. Inasmuch as the optical rotations of **2** and **3** in $CHCl_3$ solution were comparable ($[\alpha]_D$ values: +57.0 and +27.0, respectively),[18] as were the two sets of ^1H and ^{13}C NMR data for comparable centers, it appeared most likely that these two molecules had identical constitution and equal absolute configuration. Indeed, the structural relationship of these compounds was decisively demonstrated when isocyanide **2**, upon reduction with $LiAlH_4$ in THF at 25 °C, yielded **3** in 51% isolated yield.[19] On the basis of these results, it was concluded that the new molecule is (1(14)-*E*,3*S**,4*R**,7*S**,8*S**,11*R**,12*S**,13*R**)-7-methylaminoisoneoamphilecta-1(14),15-diene.

The accurate molecular mass of 7-formamidoisoneoamphilecta-1(14),15-diene (**4**) indicated it to have the molecular formula $C_{21}H_{33}NO$. From its UV and 1D/2D NMR data (see Figure 1 and Table 2), it appeared that **4** also was an isoneoamphilectane derivative containing the same conjugated diene functionality as **2** and **3** [UV(MeOH), λ_{max} 232 nm (ε 9000)]. From the IR and NMR data of **4**, it was also evident that the only differences between **2** and **4** were a result of the C-7 isocyanide functionality in **2** emerging as a formamide in **4**. The presence of the latter functionality in **4** was further supported by the existence of two sets of NMR signals in a 2:1 ratio due to the two possible geometries a formamide adopts on the NMR time scale (i.e., **4a** and **4b**; Table 2).[1,10] Close comparison of the NMR data for **4** with those for **2** and **3** led to the deduction that the formamide functionality resided at C-7 (δ_C 163.0, 57.0 [*trans*]; 160.4, 58.6 [*cis*]), showing **4** to be the 7-formamido derivative of 7-isocyanoisoneoamphilecta-1(14),15-diene (**2**). Contrary to previous reports on the geometry of the formamide group, the coupling constants of 7-NHC*H*O (δ 8.26, d, J = 12.3 Hz for the major rotamer **4a**, and δ 8.04, d, J = 2.1 Hz for the minor **4b** rotamer) clearly indicated the *transoid* conformation to be the dominant species.[3b,4] As the NMR data of **2**−**4** were virtually identical for comparable stereocenters, and the fact that the optical rotation of **4** ($[\alpha]_D$ +44.0) was of similar magnitude and sign to those of **2** and **3**, it is likely that these three molecules all have identical absolute configuration. Furthermore, hydrolysis of isocyanide **2** with 98% AcOH yielded **4** in 65% yield demonstrating conclusively that the latter compound is in fact (1(14)-*E*,3*S**,4*R**,7*S**,8*S**,11*R**,12*S**,13*R**)-7-formamidoisoneoamphilecta-1(14),15-diene.

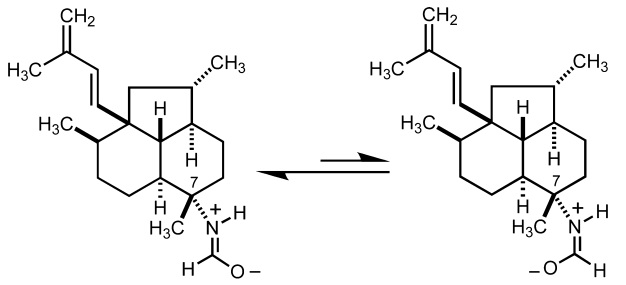
Absolute Configuration of the Tricyclic Isoneoamphilectane Core of Diterpenes 2−5. Oddly, the initial isolation of isocyanide **2** that led to the determination of its structure by extensive NMR spectroscopy studies and to the initial report of its biological activity did not provide for the structural determination of its molecular stereochemistry.[10] As the determination of the absolute configuration of chiral molecules is an important aspect of molecular stereochemistry, we decided to undertake this crucial task by using vibrational

dx.doi.org/10.1021/jo401846m | J. Org. Chem. 2013, 78, 11294−11301

Table 2. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) Spectroscopic Data for 4a and 4b[a]

	trans rotational isomer **4a** (major)		*cis* rotational isomer **4b** (minor)	
position	δ_H, mult (*J*, Hz)	δ_C, type[b]	δ_H, mult (*J*, Hz)	δ_C, type[b]
1	5.64, d (16.1)	137.1, CH	5.65, d (16.1)	137.6, CH
2α	1.02, br m	48.8, CH_2	1.02, br m	48.9, CH_2
2β	2.11, br m		2.11, br m	
3	1.42, br m	37.2, CH	1.42, br m	37.3, CH
4	0.92, br m	54.4, CH	0.98, br m	54.3, CH
5α	1.82, br m	26.9, CH_2	1.82, br m	27.1, CH_2
5β	1.19, br m		1.19, br m	
6α	1.52, br m	43.0, CH_2	1.99, br m	38.6, CH_2
6β	1.90, br m		2.12, br m	
7		57.0, C		58.6, C
8	1.47, br m	47.7, CH	2.07, br m	44.1, CH
9α	1.06, br m	19.5, CH_2	0.86, br m	19.9, CH_2
9β	1.59, br m		1.52, br m	
10α	1.59, br m	28.1, CH_2	1.59, br m	28.3, CH_2
10β	1.26, br m		1.26, br m	
11	1.56, br m	35.6, CH	1.56, br m	35.6, CH
12		46.0, C		46.1, C
13	1.27, br m	53.8, CH	1.27, br m	53.6, CH
14	6.10, d (16.1)	129.8, CH	6.10, d (16.1)	129.6, CH
15		142.2, C		142.4, C
16	4.88, br s	114.6, CH_2	4.88, br s	114.4, CH_2
17	1.82, s	18.8, CH_3	1.82, s	18.9, CH_3
18	0.91, d (6.2)	16.8, CH_3	0.90, d (6.2)	16.9, CH_3
19	1.24, s	19.3, CH_3	1.25, s	18.9, CH_3
20	0.76, d (6.2)	15.7, CH_3	0.75, d (6.2)	15.8, CH_3
21	8.26, d (12.3)	163.0, CH	8.04, d (2.1)	160.4, CH

[a]Spectra were recorded in $CDCl_3$ at 25 °C. Chemical shift values are in ppm relative to the residual $CHCl_3$ (7.26 ppm) or $CDCl_3$ (77.0 ppm) signals. [b]^{13}C NMR types were obtained from a DEPTQ experiment.



circular dichroism (VCD), an increasingly popular method for the unambiguous assignment of absolute configuration in chiral molecules.[15,20] To achieve this worthwhile endeavor, we pursued the following strategy. While all of the isoneoamphilectanes in hand could serve as bona fide candidates for VCD analysis, we chose to work with **4**, the most abundant analogue. However, as the 7-formamide functionality undergoes *cis*/*trans* isomerization that would otherwise interfere with our investigation, **4** was transformed into 7-amino derivative **5**, which does not isomerize, preserves the original chirality and requires the least amount of computational time. Thus, we begun our study by building compounds **5** and *ent*-**5** with HyperChem[21] and then conducted a conformational search for **5** at the molecular mechanics level.[22] Then the geometry optimization, frequency, IR, and VCD intensity calculations of the conformers resulting from the conformational search were carried out at the DFT level [B3LYP functional/6-31G(d) basis set][23] with Gaussian 09.[24] The calculated frequencies were scaled by 0.966 (the best scaling factor giving the best agreement between the experimental and calculated spectra)[23,25] and the IR and VCD intensities were converted to Lorentzian bands with 6-cm^{-1} half-width for subsequent comparisons to the experimental data.

The Gaussian calculations resulted in three conformers (Figure 2) that have energies within 0.4 kcal/mol from the lowest energy conformer, and all the other conformers are more than 2 kcal/mol higher than the lowest energy conformer. The optimized geometries, the relative energies, and the Boltzmann populations of the three lowest energy conformers for the 12*S* configuration are shown in Figure 2. Comparing the observed VCD and IR spectra with those of the calculated VCD of the conformers of 12*S* configuration (Figures 3 and 4A), it is clear that the chirality of C-12 is *S* based on the signs of the VCD bands. Therefore, the complete absolute configuration of **5** was determined as 3*S*,4*R*,7*S*,8*S*,11*R*,12*S*,13*R*. The assignment was also evaluated by the Compare*VOA* algorithm[25] and the confidence level of the C-12 assignment of *S* is 100% based on a current database that includes 105 previously correct assignments for different chiral structures (Figure 4B).

Bioactivity. Naturally occurring isoneoamphilectanes **2**–**4**, as well as semisynthetic derivative **5**, were evaluated against the *Mycobacterium tuberculosis* strain H_{37}Rv. Although all of the isolates were active, surprisingly, semisynthetic analogue **5** displayed the most inhibitory activity with an MIC value of 6 μg/mL (Table 3). The realization that derivative **5** is so much more active than the natural products **3** and **4**, taken together with the fact that it showed no significant cytotoxicity against

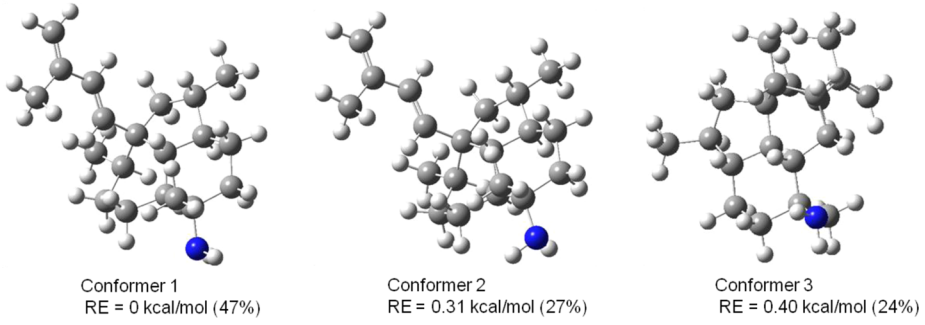


Figure 2. Optimized geometries, relative energies, and Boltzmann populations of the three calculated lowest energy 12*S* conformers of 7-aminoisoneoamphilecta-1(14),15-diene (**5**).

dx.doi.org/10.1021/jo401846m | J. Org. Chem. 2013, 78, 11294–11301

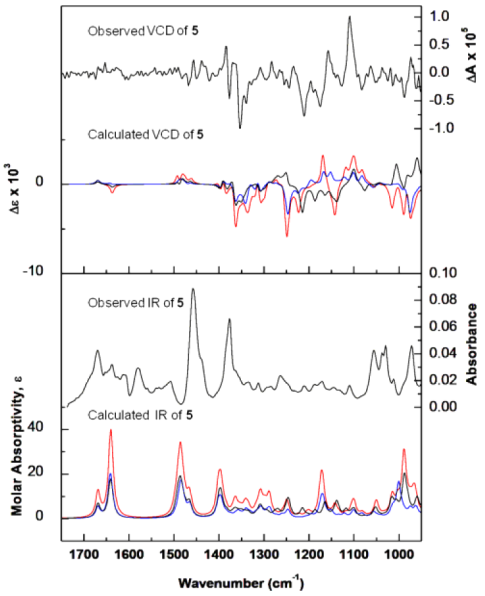


Figure 3. IR (lower frame) and VCD (upper frame) spectra observed for **5** (right axes) compared with calculated Boltzmann-population-weighted spectra of the three calculated lowest-energy conformers of the 12*S* configuration (left axes).

mammalian cells in the VERO cell assay (IC_{50} > 128 μg/mL), is clearly of some significance. Conceivably, these data suggest that the isoneoamphilectane backbone is a key pharmacophoric moiety and might represent a novel scaffold for the development of clinically useful agents for tuberculosis. With respect to the toxicological effects of these analogues on mycobacteria, research is still necessary in order to understand the mechanism of action. Unfortunately, issues associated with the availability of these compounds have prevented us from further pursuing these investigations.

Table 3. In Vitro Anti-TB Activities of Isoneoamphilectane Diterpenes 2−5 against *M. tuberculosis* H_{37}Rv and Determination of Their Cytotoxicity Against the VERO Cell Line[a]

	% inhibition in test concn (128−8 μg/mL)					MIC[b]	cytotoxicity[c]
compd	128	64	32	16	8	*Mtb* H_{37}Rv strain	VERO cells
2	100	99	99	98	89	8	19
3	99	100	100	95	7	15	N.T.
4	99	100	91	30	17	32	N.T.
5	100	100	100	100	99	6	>128
RMP[d]	100	100	100	100	99	0.05	89

[a]Values are means of three experiments. [b]Minimum inhibitory concentrations (MIC) in μg/mL. [c]IC_{50} in μg/mL. The cytotoxicity data for **2** (taken from ref 10) are against mammalian KB cells. N.T. = not tested. [d]Rifampin (RMP) was used as a positive control.

CONCLUSIONS

Arguably the most significant finding of this study was the discovery of the second and third examples of an exceptionally rare class of natural products, namely, the isoneoamphilectanes. For almost two decades, this family of metabolites has been conformed by a single member. A comprehensive listing of fully reported and assigned $^{1}H-^{13}C$ NMR data (Tables 1 and 2) for the new analogues **3** and **4** was provided, and these data should facilitate the assignment of structurally similar, yet to be discovered, natural products. Although each metabolite could be derived chemically from reduction or hydrolysis of isocyanide **2**, we did not address experimentally whether **3** and **4** are formed biosynthetically. Compounds **2**−**5** were tested for their in vitro biological activity against *M. tuberculosis* H_{37}Rv. The results of these assays (Table 3) showed only derivative **5** to have antitubercular activity that can be regarded as significant and somewhat selective, based on its lack of cytotoxicity toward VERO cells. Furthermore, our data suggest that the isoneoamphilectane backbone, and not the isocyanide functionality, may well be the molecular feature responsible for

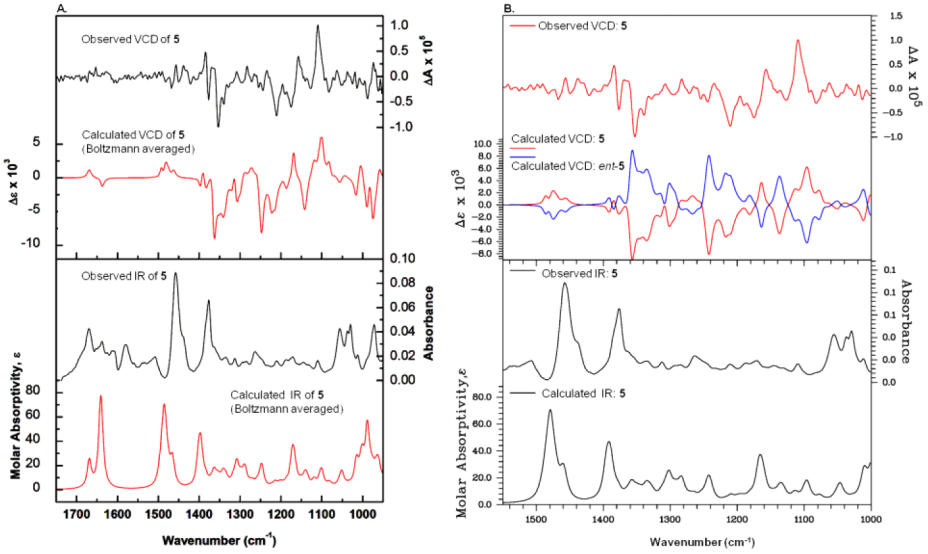


Figure 4. (A) IR (lower frame) and VCD (upper frame) spectra observed for **5** (right axes) compared with the Boltzmann-averaged calculated spectra of the three lowest energy conformations of the 12*S* configuration (left axes). (B) Compare*VOA* program showing experimental VCD and IR spectra of compound **5** compared to the theoretical calculation of both enantiomers.

dx.doi.org/10.1021/jo401846m | *J. Org. Chem.* 2013, 78, 11294−11301

the observed activity. For the first time, the absolute configuration of the isoneoamphilectane backbone has been designated as 3*S*,4*R*,7*S*,8*S*,11*R*,12*S*,13*R*. The present work can serve as another demonstration for a successful application of vibrational circular dichroism spectroscopy, establishing it as an easy method to designate the absolute configuration of suitable molecules. Future synthetic work undertaken on the basis of these natural product leads would clearly demonstrate the potential of these diterpenes as important prototype structures for new antitubercular agents. With no structural ambiguities left, many groups might now embarked on the total synthesis of these structurally challenging natural products along with different stereoisomers as targets suited to pursue structure−activity studies.[26]

EXPERIMENTAL SECTION

General Experimental Methods. Optical rotations were measured in $CHCl_3$ at 589 nm using a 10 cm microcell. IR spectra were recorded as neat films employing an FT-IR spectrometer with 4 cm^{-1} resolution. The UV spectra were recorded from 200 to 800 nm using a path length of 10 mm. Exact mass measurements were performed with a hybrid quadrupole time-of-flight mass spectrometer equipped with an electrospray (ESI) ion source. ESI samples were introduced through direct infusion into the ESI source using a syringe pump and MeOH as the solvent. For positive-ion mode, 0.1% formic acid or acetic acid was usually added into the analyte solution to enhance protonation and increase sensitivity. 1D and 2D NMR spectra were recorded at 500 MHz. ^1H and ^{13}C NMR chemical shifts are reported in ppm relative to the residual $CHCl_3$ signal (7.26 ppm) and $CDCl_3$ signal (77.0 ppm), respectively. Saturated solutions of anhydrous NH_3 in MeOH were prepared by bubbling dry gaseous NH_3 into dry MeOH. The percentage yield of each compound is based on the weight of the dry sponge specimen.

Animal Material. The Caribbean sponge *Svenzea flava* (phylum Porifera; class Demospongiae; order Halichondrida; family Dictyonellidae) was collected at a depth of 80−90 feet by scuba off Great Inagua Island, Bahamas, in July 2011. A voucher specimen (no. SV11-07) is stored at the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. The species *Svenzea flava* was originally classified as *Pseudoaxinella flava*.[13] Despite lacking dark granulous cells that are a signature characteristic of other species within the genus *Svenzea*, it has been accepted as *Svenzea flava*.[14]

Extraction and Isolation. Shortly after collection, the frozen sponge specimen was lyophilized and the dry specimen (190 g) cut into small pieces and blended with a 1:1 mixture of $CHCl_3$−MeOH (6 × 1 L). The combined extract was filtered and concentrated in vacuo to yield an orange gum (13.2 g) that was later suspended in H_2O (2 L) and partitioned against solvents of increasing polarity (*n*-hexane, $CHCl_3$, EtOAc) (4 × 500 mL). After concentration of the combined *n*-hexane extracts, the remaining orange oily residue (5.9 g) was subjected to vacuum liquid chromatography (VLC) over silica gel (150 g) using a *n*-hexane/EtOAc gradient (98:2−1:1) to give five fractions (denoted I−V) on the basis of in-house biological activity assays and routine TLC and ^1H NMR analyses. Further purification of fraction II (1.2 g) by silica gel (20.0 g) column chromatography (CC) using 2% *n*-hexane/EtOAc afforded 14 subfractions, denoted as A−N. Subfraction C was identified as the known compound 7-isocyanoisoneoamphilecta-1(14),15-diene (**2**) (12 mg, 0.006%) on the basis of IR, UV, $[\alpha]_D$, MS, and ^1H and ^{13}C NMR spectroscopic analyses.[10] Subfraction E consisted of a pure colorless crystalline solid that was subsequently identified as known (−)-8,15-diisocyano-11(20)-amphilectene (**6**) (108 mg, 0.06%) after X-ray crystallographic analysis.[1a] Similarly, subfractions F and J were subsequently identified as the known compounds ergosterol (24 mg, 0.01%)[27] and 7,15-diisocyano-11(20)-amphilectene (**8**) (37 mg, 0.02%),[2] respectively, by spectroscopic analysis and from comparisons to the known literature data. Analysis of the data obtained for subfraction H revealed that this portion consisted of new 7-methylaminoisoneoamphilecta-1(14),15-diene (**3**) (7 mg, 0.004%). Purification of subfraction L by CC over a short plug of silica gel (1.0 g) using 2% MeOH−$CHCl_3$ yielded pure 7-formamidoisoneoamphilecta-1(14),15-diene (**4**) (14 mg, 0.007%). Lastly, subfraction M was recognized as the previously known 8-isocyano-11(20)-ene-15-amphilectaformamide (**7**) (46 mg, 0.02%).[1a]

*7-Methylaminoisoneoamphilecta-1(14),15-diene (**3**):* colorless oil; $[\alpha]^{20}_D$ +27.0 (*c* 1.0, $CHCl_3$); UV (hexane) λ_{max} (log ε) 234 (4.2) nm; IR (film) ν_{max} 3404, 3078, 3022, 2949, 2925, 2856, 2796, 1639, 1606, 1475, 1456, 1375, 1170, 1109, 970, 881 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 1); HRMS (ESI) $[M + H]^+$ *m*/*z* 302.2856 (calcd for $C_{21}H_{36}N$, 302.2848).

*7-Formamidoisoneoamphilecta-1(14),15-diene (**4**):* colorless oil; $[\alpha]^{20}_D$ +44.0 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (3.9) nm; IR (film) ν_{max} 3296, 3053, 2947, 2927, 2868, 1679, 1535, 1456, 1377, 970, 885, 756 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) (see Table 2); HRMS (ESI) $[M + H]^+$ *m*/*z* 316.2651 (calcd for $C_{21}H_{34}NO$, 316.2640).

Reduction of 7-Isocyanoisoneoamphilecta-1(14),15-diene (2) with $LiAlH_4$. Isocyanide **2** (2.3 mg, 0.008 mmol) in anhydrous THF (0.5 mL) was added slowly to a suspension of $LiAlH_4$ (1.2 mg, 0.03 mmol) in THF (0.5 mL) under argon. The reaction mixture was stirred for 20 h at 25 °C before it was quenched with 1 mL of MeOH. After the resulting slurry was filtered through a short plug of Celite, $CHCl_3$ (5 mL) was added and the solution washed with brine, dried, concentrated, and purified by CC on silica gel (1.0 g, 99:1 (v/v) MeOH in $CHCl_3$) to give 1.2 mg (51%) of **3**; all solution spectral and TLC data were identical with those obtained from authentic material.

Basic Hydrolysis of 7-Formamidoisoneoamphilecta-1(14),15-diene (4). A mixture of formamide **4** (6 mg, 0.02 mmol) and KOH 1 M (5 mL) in freshly distilled MeOH (10 mL) was slowly heated to 60 °C with constant stirring for 12 h. After being cooled to rt, the reaction mixture was concentrated in vacuo, and the oily residue obtained suspended in H_2O (5 mL) and extracted with $CHCl_3$ (3 × 5 mL). The combined organic layer was dried ($MgSO_4$), filtered, and concentrated to leave a residue that was purified over silica gel (300 mg) using a mixture of 2% MeOH(NH_3) in $CHCl_3$ to afford 7-aminoisoneoamphilecta-1(14),15-diene (**5**) (4.9 mg, 90% yield): colorless oil; $[\alpha]^{20}_D$ +28.0 (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (4.0) nm; IR (film) ν_{max} 3445, 3354, 2950, 2925, 2868, 1639, 1606, 1566, 1456, 1365, 970, 881 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.11 (d, 1H, *J* = 16.1 Hz, H-14), 5.67 (d, 1H, *J* = 16.1 Hz, H-1), 4.89 (br s, 2H, H-16), 2.12 (m, 1H, H-2), 1.84 (s, 3H, H-17), 1.79 (m, 1H, H-5), 1.70 (m, 1H, H-6), 1.69 (m, 1H, H-9), 1.60 (m, 1H, H-10), 1.58 (m, 1H, H-11), 1.42 (m, 1H, H-3), 1.31 (m, 1H, H-6′), 1.28 (m, 1H, H-8), 1.27 (m, 1H, H-10′), 1.23 (m, 1H, H-13), 1.14 (m, 1H, H-5′), 1.02 (m, 1H, H-2′), 1.01 (m, 1H, H-9′), 1.00 (s, 3H, H-19), 0.91 (d, 3H, *J* = 6.4 Hz, H-18), 0.90 (m, 1H, H-4), 0.76 (d, 3H, *J* = 6.4 Hz, H-20); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 142.5 (C, C-15), 137.9 (CH, C-1), 129.4 (CH, C-14), 114.1 (CH_2, C-16), 55.0 (CH, C-4), 54.9 (CH, C-13), 53.2 (C, C-7), 49.1 (CH, C-8), 49.0 (CH_2, C-2), 46.1 (C, C-12), 44.3 (CH_2, C-6), 37.3 (CH, C-3), 35.7 (CH, C-11), 28.6 (CH_2, C-10), 27.7 (CH_2, C-5), 21.6 (CH_3, C-19), 19.6 (CH_2, C-9), 18.9 (CH_3, C-17), 17.0 (CH_3, C-18), 15.9 (CH_3, C-20); HRMS (ESI) $[M + H]^+$ *m*/*z* 288.2692 (calcd for $C_{20}H_{34}N$, 288.2691).

Acid Hydrolysis of 7-Isocyanoisoneoamphilecta-1(14),15-diene (2). A homogeneous mixture of isocyanide **2** (10 mg, 0.031 mmol) and glacial AcOH (3 drops) dissolved in freshly distilled MeOH (10 mL) was stirred at 25 °C for 24 h. After concentration, the resulting solid residue left over was purified by flash CC over silica gel using 2% MeOH(NH_3)−$CHCl_3$ to afford 7.2 mg (65%) of 7-formamidoisoneoamphilecta-1(14),15-diene (**4**).

VCD Analysis and DFT Calculations. Semisynthetic derivative 7-aminoisoneoamphilecta-1(14),15-diene (**5**) (3.8 mg) was dissolved in $CDCl_3$ (3.8 mg/0.15 mL) and placed in a 100 μm path-length IR cell with BaF_2 windows. IR and VCD spectra were recorded on a Chiral*IR2X* VCD spectrometer (BioTools, Inc.) equipped with dual photo elastic modulator (PEM) accessory with 4 cm^{-1} resolution, 20 h collection for both the sample and $CDCl_3$, and the two PEMs instruments optimized at 1400 cm^{-1}. Both the IR and the VCD baselines were obtained by subtracting the solvent spectra from the

dx.doi.org/10.1021/jo401846m | J. Org. Chem. 2013, 78, 11294−11301

sample spectra. The geometry optimization, IR and VCD intensities of all the conformers generated by conformational search using HyperChem were calculated with a Linux AMD64 2.4 GHz using the Gaussian 09.[24] The comparison between the observed and the calculated spectra were evaluated by the Compare*VOA* program developed by BioTools.[25,28]

Antitubercular Activity Bioassays. The antitubercular activity of isoneoamphilectane diterpenes **2**–**5** was evaluated against the laboratory strain *Mycobacterium tuberculosis* H_{37}Rv. A detailed description of the experimental methods used has been previously described.[29] All of the antitubercular assays were performed at the Institute for Tuberculosis Research, University of Illinois at Chicago, College of Pharmacy, Chicago, IL, by members of Professor Scott G. Franzblau's Research Group.

■ ASSOCIATED CONTENT

Supporting Information

Underwater photograph of *S. flava*, copies of the ^{1}H NMR, ^{13}C NMR, 2D NMR, and ESI-MS spectra for isoneoamphilectane diterpenes **3**–**5**, coordinates and energies for all computed structures (Tables 4–6), and plausible biosynthetic pathways. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: abimael.rodriguez1@upr.edu

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Dr. Scott G. Franzblau and Dr. Yuehong Wang from the Institute for Tuberculosis Research (University of Illinois at Chicago) for the antituberculosis bioactivity data and Dr. Yanan He (BioTools, Inc., Jupiter, FL) for providing the calculated and experimental VCD spectra for compound **5**. Mass spectroscopic determinations were provided by the Mass Spectrometry Laboratory of the University of Illinois at Urbana–Champaign. Financial support to E. Avilés was generously provided by the GK-12 Fellowship (Grant No. 0841338). We thank Dr. Joseph R. Pawlik (UNC–Wilmington) for graciously allowing one of us (J.V.) to participate in an expedition to the Bahamas, during which the sponge *S. flava* was collected. This research was supported by a grant from the NIH-SC1 Program (Grant 1SC1GM086271-01A1) awarded to A.D. Rodríguez.

■ REFERENCES

(1) (a) Wratten, S. J.; Faulkner, D. J.; Hirotsu, K.; Clardy, J. *Tetrahedron Lett.* **1978**, 4345–4348. (b) Avilés, E.; Rodríguez, A. D. *Org. Lett.* **2010**, *12*, 5290–5293.

(2) (a) Ciavatta, M. L.; Fontana, A.; Puliti, R.; Scognamiglio, G.; Cimino, G. *Tetrahedron* **1999**, *55*, 12629–12636. (b) Ciavatta, M. L.; Gavagnin, M.; Manzo, E.; Puliti, R.; Mattia, C. A.; Mazzarella, L.; Cimino, G.; Simpson, J. S.; Garson, M. J. *Tetrahedron* **2005**, *61*, 8049–8053.

(3) (a) Mitome, H.; Shirato, N.; Miyaoka, H.; Yamada, Y.; Van Soest, R. W. M. *J. Nat. Prod.* **2004**, *67*, 833–837. (b) Chanthathamrongsiri, N.; Yuenyongsawad, S.; Wattanapiromsakul, C.; Plubrukarn, A. *J. Nat. Prod.* **2012**, *75*, 789–792. (c) Wattanapiromsakul, C.; Chanthathamrongsiri, N.; Bussarawit, S.; Yuenyongsawad, S.; Plubrukarn, A.; Suwanborirux, K. *Can. J. Chem.* **2009**, *87*, 612–618.

(4) (a) Linden, A.; König, G. M.; Wright, A. D. *Acta Crystallogr.* **1996**, *C52*, 2601–2607. (b) Wright, A. D.; Lang-Unnasch, N. *J. Nat. Prod.* **2009**, *72*, 492–495.

(5) (a) Baker, J. T.; Wells, R. J.; Oberhansli, W. E.; Hawes, G. B. *J. Am. Chem. Soc.* **1976**, *98*, 4010–4012. (b) Kazlauskas, R.; Murphy, P. T.; Wells, R. J.; Blount, J. F. *Tetrahedron Lett.* **1980**, *21*, 315–318.

(6) Molinski, T. F.; Faulkner, D. J.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1987**, *52*, 3334–3337.

(7) Ciasullo, L.; Cutignano, A.; Casapullo, A.; Puliti, R.; Mattia, C. A.; Debitus, C.; Riccio, R.; Gomez-Paloma, L. *J. Nat. Prod.* **2002**, *65*, 1210–1212.

(8) Lamoral-Theys, D.; Fattorusso, E.; Mangoni, A.; Perinu, C.; Kiss, R.; Costantino, V. *J. Nat. Prod.* **2011**, *74*, 2299–2303.

(9) Sharma, H. A.; Tanaka, J.; Higa, T.; Lithgow, A.; Bernardinelli, G.; Jefford, C. W. *Tetrahedron Lett.* **1992**, *33*, 1593–1596.

(10) König, G. M.; Wright, A. D.; Angerhofer, C. K. *J. Org. Chem.* **1996**, *61*, 3259–3267.

(11) Blunt, J. W.; Copp, B. R.; Keyzers, R. A.; Munro, M. H. G.; Prinsep, M. R. *Nat. Prod. Rep.* **2013**, *30*, 237–323 and previous articles in this series.

(12) (a) Wright, A. D.; Wang, H.; Gurrath, M.; König, G. M.; Kocak, G.; Neumann, G.; Loria, P.; Foley, M.; Tilley, L. *J. Med. Chem.* **2001**, *44*, 873–885. (b) Gross, H.; König, G. M. *Phytochem. Rev.* **2006**, *5*, 115–141. (c) Wright, A. D.; McCluskey, A.; Robertson, M. J.; MacGregor, K. A.; Gordon, C. P.; Guenther, J. *Org. Biomol. Chem.* **2011**, *9*, 400–407. (d) Mayer, A. M. S.; Avilés, E.; Rodríguez, A. D. *Bioorg. Med. Chem.* **2012**, *20*, 279–282.

(13) Lehnert, H.; Van Soest, R. W. M. *Beaufortia* **1999**, *49*, 141–169.

(14) Alvarez, B.; Van Soest, R. W. M.; Rützler, K. *Contrib. Zool.* **2002**, *71*, 171–176.

(15) (a) He, Y.; Wang, B.; Dukor, R. K.; Nafie, L. A. *Appl. Spectrosc.* **2011**, *65*, 699–722. (b) Felippe, L. G.; Batista, J. M.; Baldoqui, D. C.; Nascimento, I. R.; Kato, M. J.; He, Y.; Nafie, L. A.; Furlan, M. *Org. Biomol. Chem.* **2012**, *10*, 4208–4214.

(16) A small amount of the well-known sterol ergosterol was also isolated.

(17) Rodríguez, A. D.; Rodríguez, I. I. *Tetrahedron Lett.* **2002**, *43*, 5601–5604.

(18) Our sample of 7-isocyanoisoneoamphilecta-1(14),15-diene (**2**) had $[\alpha]^{20}_{D}$ +57.0 (*c* 1.0, $CHCl_3$). In the original study (ref 10), **2** had $[\alpha]^{25}_{D}$ +67.0 (*c* 0.79, $CHCl_3$).

(19) Van Leusen, D.; Van Leusen, A. M. *Org. React.* **2001**, *57*, 417–679.

(20) While X-ray crystallography is a straightforward and highly reliable method for establishing absolute configuration, this method requires a single crystal of the sample of sufficient size and quality. Moreover, an atom heavier than oxygen must generally be present in the molecule (or must be incorporated into the molecule by derivatization) in order to determine the absolute configuration of the crystal unambiguously (for an excellent review of the use and limitations of X-ray crystallography to determine absolute configuration, see: Flack, H. D.; Bernardinelli, G. *Chirality* **2008**, *20*, 681–690). Because none of the present isoneoamphilectanes met these requirements, we chose VCD. This method is the extension of electronic CD into the infrared region, where fundamental vibrational transitions occur. VCD has a number of advantages over the other methods of absolute configuration assignment but has some limitations as well. Like X-ray crystallography, VCD is an absolute, not a relative, method, but unlike X-ray analysis does not require the presence of a heavy atom in the molecule and is not restricted to single crystals. VCD spectra are recorded for neat liquid or solution in suitable solvents (CS_2, CCl_4, $CDCl_3$), and no derivatization of the molecule is required prior to analysis.

(21) Hypercube, Inc., 1115 NW 4th St, Gainesville, FL 32601.

(22) We used the MMFF94 molecular mechanics force-field for the conformational search.

(23) (a) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 1372–1377. (b) Kim, K.; Jordan, K. D. *J. Phys. Chem.* **1994**, *98*, 10089–10094. (c) Stephens, P. J.; Devlin, F. J.; Chabalowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623–11627.

(24) Gaussian 09, Revision A.1: Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.;

Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A., Jr.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J. M.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. Gaussian, Inc., Wallingford, CT, 2009.

(25) Debie, E.; De Gussem, E.; Dukor, R. K.; Herrebout, W.; Nafie, L. A.; Bultinck, P. *Chem. Phys. Chem.* **2011**, *12*, 1542−1549.

(26) For an insightful application of intramolecular Diels−Alder cycloadditions of fulvenes to a variety of polycyclic rings, including the isoneoamphilectane skeleton, see: Hong, B.-C.; Chen, F.-L.; Chen, S.-H.; Liao, J.-H.; Lee, G.-H. *Org. Lett.* **2005**, *7*, 557−560.

(27) (a) Chobot, V.; Opletal, L.; Jahodar, L.; Patel, A. V.; Dacke, C. G.; Blunden, G. *Phytochemistry* **1997**, *45*, 1669−1671. (b) Lee, D. I.; Choi, J. S.; Yang, M. R.; Lee, W. K.; Kim, D. S.; Choi, H. D.; Son, B. W. *Nat. Prod. Sci.* **1999**, *5*, 93−96.

(28) BioTools, Inc., 17546 Beeline Highway, Jupiter, Florida 33458.

(29) Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004−1009.