A New Cycloamphilectene Metabolite from the Vanuatu Sponge *Axinella* sp.

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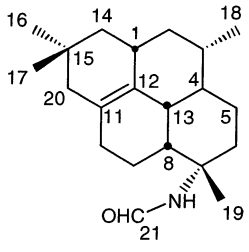
A new diterpene, *N*-formyl-7-amino-11-cycloamphilectene (**1**), was isolated from the apolar extract of the Vanuatu sponge *Axinella* sp. The structure and relative stereochemistry were established by spectroscopic and single-crystal X-ray studies.

Diterpenes based on amphilectene and cycloamphilectene skeletons have usually been found in sponges belonging to the order Halichondrida,[1−4] although there are recent papers on these metabolites from taxonomically different sponges.[5,6] These classes of metabolites are usually characterized by poorly functionalized tri- or tetracyclic carbon frameworks, but in most cases one or more isonitrile, isocyanate, or isothiocyanate functions are present. Moreover, these substances show significant antimalarial, cytotoxic, or antimicrobial biological activities.

In our continuing study on marine metabolites from sponges, we isolated from *Axinella* sp. collected off the coasts of Vanuatu Islands compound **1**, *N*-formyl-7-amino-11-cycloamphilectene. This new compound is the formamide that logically derives from 7-isocyano-11-cycloamphilectene, a metabolite previously isolated and characterized by Molinski et al.[3] from the Palauan sponge *Halichondria* sp.

The freeze-dried organism was initially extracted with methanol. The cytotoxic nonpolar fractions, obtained according to the Kupchan extraction procedure,[7] were chromatographed by MPLC on silica gel (*n*-hexane/EtOAc gradient) and then by HPLC on an ODS-2 column (MeOH/H_2O, 9:1), affording **1** as a pure crystalline compound (73.5 mg).



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Structural analysis of **1** was achieved in part on the basis of ESI-MS and NMR (mono- and bidimensional) data. The ESI-MS spectrum of **1** contained peaks at *m/z* 316.4, 338.3, and 653.3, which were attributed to pseudomolecular ions $[M + H]^+$, $[M + Na]^+$, and $[2M + Na]^+$ all pointing to a MW of 315 amu. NMR spectra were indicative of an apolar and moderately functionalized carbon framework. The ^1H NMR spectrum ($CDCl_3$), which was very crowded in the high-field region, contained resonances belonging to two different sets of signals, likely due to a conformational equilibrium slower than the NMR time scale. Accordingly, the two spin systems downfield shifted at δ 5.64−8.27 and 5.15−8.12, respectively, were attributed, on the basis of a COSY experiment, to conformational isomerism between *cis* and *trans* orientations of the NH-CHO group. The only other functional group of the molecule was a tetrasubstituted double bond, the presence of which was established by the sp^2 carbon signals at 125.4 and 133.0 ppm. Because most signals in the region between 0.8 and 2.2 ppm were severely overlapped, we utilized HSQC-TOCSY for a complete assignment of all resonances of the spectrum, along with traditional TOCSY, HSQC, and HMBC experiments (Table 1).

Table 1. NMR Data for **1** in $CDCl_3$[a]

no.	^1H, mult., *J* (Hz)	^{13}C	HMBC
1	2.03, m	36.1	
2	0.75,m; 1.81, m	46.0, *45.9*	C-18
3	1.33, m; *2.33, m*	38.2, *41.5*	C-5
4	0.96 m	44.5, *44.2*	C-18
5	1.15,m, 1.85 m; *1.14, m; 1.78, m*	27.5, *25.5*	C-3, C-6
6	1.56, m, 1.61, m; *1.51, m, 1.75, m*	33.0, *33.7*	C-7
7		55.5	
8	1.63m; *0.96, m*	46.0, *44.5*	C-7, C-19
9	1.45, m, 1.68, m; *1.29, m, 1.55, m*	19.9, *20.0*	C-7
10	1.87, m, 1.93, m	32.0, *31.9*	C-8
11		125.4, *125.3*	
12		133.0, *132.8*	
13	1.80, m; *1.76, m*	42.0, *41.5*	
14	0.86, m, 1.41, m	44.7, *44.6*	C-1
15		29.3	
16	0.81, s; *0.80, s*	24.7, *24.8*	C-15, C-17
17	0.92, s; *0.91, s*	31.7, *31.8*	C-15, C-16, C-20
18	0.87, d (*6.14*)	19.3	C-2, C-3, C-4
19	1.45, s; *1.54, s*	27.4	C-6, C-7, C-8
20	1.50, m, 1.75, m	44.1, *44.3*	C-15, C-16, C-17
21	8.27, d, (*12.2*); *8.09, bs*	163.0, *160.1*	
NH	5.64, d, (*12.2*); 5.15, bs		

[a] Chemical shift values in italics refer to the *cis* conformer.

All structural features of **1**, including the relative stereochemistry (1*S**,3*S**,4*R**,7*S**,8*R**,13*R**), were clarified by an X-ray diffraction study on a single crystal obtained by careful recrystallization from a ethanol/ethyl ether solution. The structure refinement on 1554 observed reflections and

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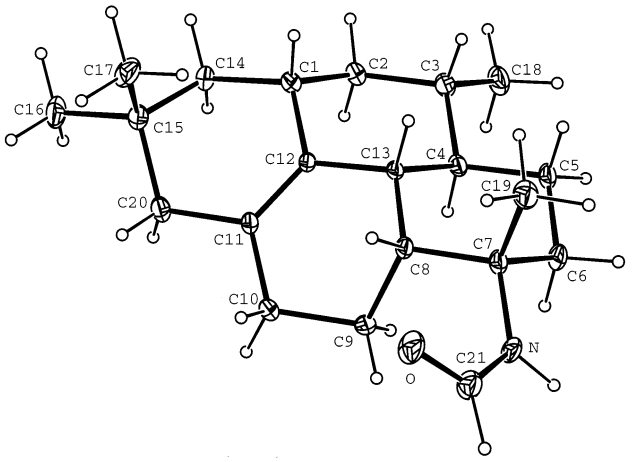

Figure 1. Perspective view of the final X-ray model of **1** with the atomic labeling for non-H atoms. Displacement ellipsoids are drawn at the 25% probability level. No absolute configuration is implied.

208 variables led to a discrepancy index $R = 0.046$. Final atomic parameters are available as Supporting Information. A perspective view of the final crystallographic model of *N*-formyl-7-amino-11-cycloamphilectene is given in Figure 1. In the absence of atoms with a strong anomalous scattering contribution, reliable evidence of the absolute stereochemistry could not be determined. The enantiomer shown was chosen arbitrarily and agrees with that of 7-isocyano-11-cycloamphilectene.[3] All the bond lengths and angles (Supporting Information Table S2) are in the expected ranges.[2,3,8−10] This diterpenoid presents a tetracyclic carbon skeleton characterized by an uncommon *cis*-junction along the C8 and C13 bond and a double bond between the 11,12-positions. Among the four methyl group substituents, the methyl at C3 is in equatorial orientation, while that at C7 is axial and on the same side as H8 and H13. The equatorial N-formyl substitution at C7 is strictly planar, with C21 *trans* to the C6 ring carbon and the carbonyl group eclipsed to the N−C7 bond: torsion angles C6− C7−N−C21 = −177.8(6)° and C7−N−C21−O = −1.3-(6)°. The two cyclohexene rings are in the expected chair conformations, and both cyclohexanes adopt half-chair conformations slightly distorted toward twist-boat forms.

In the crystal (Figure 1), molecules related by *screw* symmetry are joined by means of hydrogen bonds involving the N-formyl groups: NO($1 - x$, $1/2 + y$, $1 - z$) = 2.980(3) Å, HNO = 2.06 Å, N−HO = 161°. All the remaining intramolecular contacts are greater than the sum of van der Waals radii.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were determined on a Bruker DRX-600 Avance spectrometer, and the solvent was used as internal standard ($CDCl_3$: ^{1}H δ 7.27, ^{13}C 77.0 ppm). 2D experiments (COSY, HSQC, HMBC, and HSQC-TOCSY) were recorded using conventional pulse sequences. Mass spectra were recorded on a Thermoquest LCQ-DECA spectrometer equipped with an electrospray source.

Biological Material. The sponge *Axinella* sp. was collected from the Vanuatu Islands (depth −14 m) and taxonomically identified by Dr. John Hooper (voucher specimen located at Museum of Queensland, Brisbane, Australia; accession number G306878).

Extraction and Isolation. The organism (lyophilized material, 670 g) was extracted exhaustively with MeOH (4 × 1 L) at room temperature. The methanolic extract, filtered through paper and concentrated under reduced pressure, gave a brown oil. The oily residue was successively extracted using a modified Kupchan partition procedure: the extract was dissolved in 1 L of a mixture of MeOH/H_2O containing 10% of H_2O and partitioned against 1 L of *n*-hexane. The water content (% v/v) of the methanolic fraction was adjusted to 20% and 40% and partitioned against 1 L of CCl_4 and 1 L of $CHCl_3$, respectively. The aqueous phase was concentrated to remove MeOH and then extracted with *n*-butanol (1 L). The CCl_4-soluble material (11.10 g) was chromatographed by medium-pressure liquid chromatography (MPLC) on a silica gel column (230−400 mesh) using a gradient elution system of *n*-hexanes/EtOAc from 100% *n*-hexanes to 100% EtOAc (30 mL/fraction). The collected fractions were analyzed by TLC on silica gel (Merck, kieselgel F_{254}, 0.25 mm) as shown by spraying with $Ce(SO_4)_2$ in sulfuric acid solution. Homogeneous fractions were pooled into 14 groups. The collected fractions 144−164 were further purified by RP-HPLC on an ODS-2 (250 × 10 mm) column, eluted with MeOH/H_2O (90:10) to afford pure compound **1** (73.5 mg).

***N*-Formyl-7-amino-11-cycloamphilectene (1):** colorless crystals; $[\alpha]^{20}_D$ 36.8° (*c* 0.4, $CHCl_3$); IR ($CHCl_3$) ν_{max} 3304, 2919, 1461, 760, 1679 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; ESIMS *m*/*z* 316.4 $[M + H]^+$, 338.3 $[M + Na]^+$, 653.3 $[2M + Na]^+$.

Crystal Structure Analysis of 1. Single crystals were obtained by slow evaporation from an ethanol and ethyl ether mixture in the form of colorless prisms. A sample of size 0.23 × 0.12 × 0.08 mm^3 was selected for the crystallographic study. All diffraction measurements were performed at room temperature (293 K) using an Enraf-Nonius CAD-4F diffractometer (software 1989)[11] and graphite-monochromated Cu Kα radiation (λ = 1.5418 Å). Accurate cell parameters were determined by least-squares refinement of the setting angles of 25 reflections at medium θ (22° < θ < 27.5°).

Crystal Data: a = 8.121(6) Å, b = 9.394(4) Å, c = 13.286-(9) Å, β = 106.88(3)°, V = 970(1) Å^3, monoclinic system, space group $P2_1$, Z = 2, $C_{21}H_{33}NO$, M_r = 315.5, D_c = 1.08 Mg·m^{-3}.

Data Collection and Structure Refinement. A total of 2127 independent reflections (θ_{max} = 75°; $0 \le h \le 10$, $0 \le k \le 11$, $-16 \le l \le 15$) were collected using $\omega-\theta$ scans as suggested by peak-shape analysis. The crystal and equipment stabilities were checked by the intensities of three standard reflections monitored every 5 h. No significant intensity decay was observed (4% variation). The intensities were corrected for Lorentz and polarization factors, but not for absorption effect (μ = 0.492 mm^{-1}).

The structure was solved by direct methods using the SIR92 package.[12] The refinement (on F) was carried out by full matrix least-squares method on the positional and anisotropic temperature parameters of the non-hydrogen atoms. All hydrogens were observed in difference Fourier maps and included at idealized positions in the final refinements, as fixed atoms, with U_{iso} set equal to U_{eq} of the respective parent atom. The distances were constrained to be C−H = 1.00, N−H = 0.95 Å. Final discrepancy index R was 0.046 on 1554 observed reflections with $I \le \sigma(I)$ and 208 variables. R_w = 0.046 with $w^{-1} = \sigma^2(F_o) + (0.015F_o)^2 + 0.05$. Residual electron densities were within the range −0.14 to 0.13 e·Å^{-3}.

All calculations were performed on a MicroVAX 3100 computer using the Enraf-Nonius (1985) SDP programs.[13] Final atomic parameters, complete molecular geometry, and structure factors have been deposited as Supporting Information.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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