Novel Potent Antimalarial Diterpene Isocyanates, Isothiocyanates, and Isonitriles from the Tropical Marine Sponge *Cymbastela hooperi*

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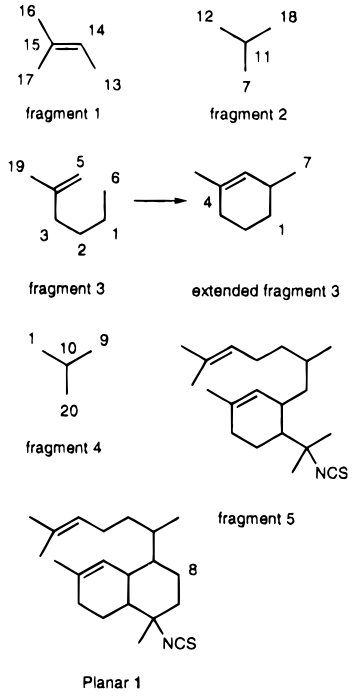
The isolation and structural elucidation of 15 diterpenes (**1**−**15**) which contain isocyanate, isothiocyanate, and isonitrile functionalities are reported from the tropical marine sponge *Cymbastela hooperi*. Of the 15, 12 (**1**, **3**−**9**, **11**, and **13**−**15**) are new natural products, two of which (**4** and **5**) contain the rare isocyanate functionality. With the exception of compounds **1** and **15**, all of the compounds are based on amphilectane, cycloamphilectane, or modified cycloamphilectane (isocycloamphilectane) ground structures. Compound **1** is an "extended" sesquiterpene, with **15** representing the first of a new class of tricyclic marine-derived diterpenes. All structures were established by spectroscopic methods, particularly $^1H-^1H$ and $^1H-^{13}C$ shift correlated 2D NMR spectroscopy and accurate mass measurement (HREIMS). All isolates with the exception of **1** demonstrated significant and selective *in vitro* antimalarial activity.

Introduction

Since our original discovery of the antimalarial activity of axisonitrile-3,[1] much of our research effort has focused on the isolation of compounds known to contain isonitrile and related funtionalities to determine whether other marine-derived natural products would also demonstrate a similar activity. As a result of these investigations, a series of novel/new marine metabolites, 12 in all (**1**, **3**−**9**, **11**, and **13**−**15**), have been isolated from a sponge, *Cymbastela hooperi* (Axinellidae, Halichondrida).[2] Of these, two (**4** and **5**) contain the rare isocyanate functionality and one (**15**) is the first member of a new class of tricyclic marine-derived diterpenes. In this paper the details of the isolation and structure elucidation of all these new compounds as well as that of the three previously reported compounds (**2**,[3,4] **10**,[5] and **12**[5]) is discussed together with their *in vitro* antimalarial and cytotoxicity data.

Compound 1. The molecular formula of **1** was determined to be $C_{21}H_{33}NS$ by accurate mass measurement. From the IR and ^{13}C NMR spectra of **1**, it was evident that the molecule contained an isothiocyanate function attached to a quaternary carbon (2075 cm^{-1}, broad; 129.7 (br), 64.7 (s) ppm), thus accounting for two of the six degrees of unsaturation indicated by the molecular formula. As there were only four further resonances in the ^{13}C NMR spectrum of **1** for carbon atoms associated with multiple bonds (121.3 (d), 124.6 (d), 131.4 (s), 135.2 (s) ppm), it was evident that the molecule was bicyclic. After all proton and carbon resonances had been associated from the results of a $^1H-^{13}C$ shift correlated 2D NMR measurement (HMQC, J = 150 Hz), it was possible to deduce the planar structure of **1** from the results contained in its $^1H-^1H$ COSY90 and $^1H-^{13}C$ HMBC (J = 8.3 Hz) spectra. Thus, the two allylic methyl groups, CH_3-16 and CH_3-17, both showed $^1H-^1H$ coupling to H-14, which further coupled to H_2-13 (fragment 1, Scheme 1). Coupling was also evident between H_3-18 and H-11, which coupled to both H_2-12 and H-7 (fragment 2, Scheme 1). The only other obvious and

Scheme 1



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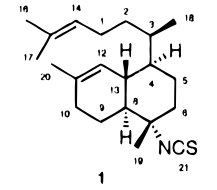
unambiguous $^1H-^1H$ couplings were evident between H_3-19 and H-5, which coupled to H_2-3, which in turn coupled to H_2-2, which demonstrated coupling to H-1, which in turn coupled to H-6 (fragment 3, Scheme 1). All of the aforementioned connectivities were substantiated by the data contained in the HMBC spectrum of **1**. From this spectrum it was also evident, from the cross peak between H_2-13 and C-12, that C-12 was adjacent to C-13, thus uniting fragments 1 and 2, Scheme 1. Further cross peaks observed between H-5 and C-1, C-6 and C-7, allowed fragment 3 to be extended and united with fragment 2. Fragment 4 was generated from the cross peaks observed between H_3-20 and C-10 (point of attachment of −NCS), C-1 and C-9. Clearly this latter fragment can be united with extended fragment 3 on the basis of the common C-1 atom to generate fragment 5. It is apparent from this fragment that the outstanding methylene group H_2-8 must reside between C-7 and C-9, thus completing the planar structure of **1**. On the basis of this information a literature search was undertaken to see if any other compounds had this carbon skeleton and a functional group at C-10. The results of this search produced three compounds that were considered of interest. The first of these, 10-isothiocyanatobiflora-4,15-diene[6] has a planar structure identical to that proposed for **1**. Close inspection of its ^{13}C NMR data and optical rotation clearly reveal, however, that this compound and **1** are different stereochemically. The two remaining compounds, dictyotins B and C,[7] also had identical planar structures to that of **1**, but each had a hydroxyl function at C-10 instead of the −NCS function found in **1**, the only difference between the two being the configuration at this center. Comparison of their ^1H and ^{13}C NMR data (recorded in pyridine-d_5) with those for **1** (recorded in $CDCl_3$) showed unequivocally that **1** must have the two six-membered rings *trans* fused and that the relative configurations at C-7 and C-3 must also be identical to those in dictyotins B and C. The one remaining center, C-10, was concluded to have the same relative configuration as that found in dictyotin B on the basis of the ^{13}C NMR chemical shift of C-20. Although the data in the NOESY spectrum of **1** could not be used to unambiguously assign any relative stereochemistry due to signal overlap, they are consistent with what has been proposed. As the optical rotation of **1** (+45.0°) is of a similar magnitude to that of dictyotin B (−30.0°) but has the opposite sign, it is proposed that **1** is the optical antipode of dictyotin B. For **1** the trivial name (1*S**,6*R**,7*R**,10*S**,11*R**)-10-isothiocyanatobiflora-1,14-diene is proposed. There are two numbering systems used for **1**. The one on the left is that used for the NMR data so that it can be easily compared with those of the other compounds in this paper, and the one on the right is the preferred numbering system applied to this type of compound. In the Discussion section the preferred numbering system is used.



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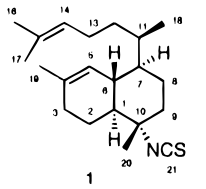
Numbering used in Tables 3 and 4 for convenient comparison with **2**-**15**



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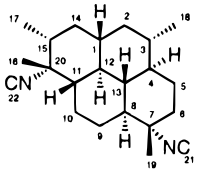
Actual numbering of this type of ring system, and the numbering used in the discussion and naming.

Modified Cycloamphilectane (Isocycloamphilectane)-Based Isolates (2−6). Compound 2 (Diisocyanoadociane). After the recording of the ^1H and ^{13}C NMR (CPD ^1H decoupled and DEPT) spectra of **2**, it was evident that the molecule was a tetracyclic diterpene, consistent with the molecular formula $C_{22}H_{32}N_2$, which contained two isonitrile functions (60.3 (br s), 64.9 (br s), 152.5 (br s), 155.3 (br s), (all four resonances demonstrate CN couplings (≈4.5 Hz) indicative of the isonitrile function)). On the basis of these data and the optical rotation and melting point of **2**, a literature search was undertaken. The results of this search suggested **2** to be diisocyanoadociane.[3,4] The general absence of NMR data in the original report[3] for this compound and unassigned incomplete data in a later report[4] made confirmation of this, at best, tentative. Thus, to check the structural deduction, as well as to provide detailed NMR data for this unusual and extremely biologically active natural product, a detailed NMR investigation of **2** was made, the results of which have been recently published.[8] For this compound we propose the new semisystematic name (1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-7,20-diisocyanoisocycloamphilectane (see Comment on Naming).



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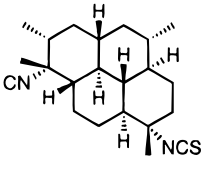

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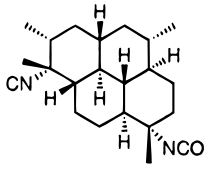

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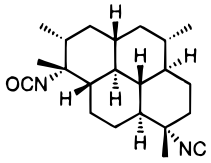

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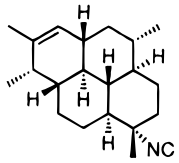

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Compound 3. From the EIMS data of **3**, it was evident that it had the molecular formula $C_{22}H_{32}N_2S$. When its ^1H and ^{13}C NMR data had been recorded and assigned (see Tables 3 and 4), it was obvious that **3** was essentially identical to **2** with the exception of the substituent at C-7. This substituent, on the basis of IR, UV, EIMS, and ^{13}C NMR spectral data, clearly had to be an isothiocyanate function. Stereochemically **3** is proposed to be identical to **2** on the basis of the almost identical ^{13}C NMR data and optical rotation comparisons (see Tables 2 and 3). For compound **3** the semisystematic name of (1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-20-isocyano-7-isothiocyanatoisocycloamphilectane is proposed.

Compound **3** is the first representative of isocycloamphilectane-based compounds to contain both isonitrile and isothiocyanate functionalities within the same molecule.

Compound 4. By accurate mass measurement **4** was found to have the molecular formula $C_{22}H_{32}N_2O$. After its ^1H and ^{13}C NMR data had been assigned, by com-

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Table 1. Methods Employed for the Isolation of Compounds 1−15

compd	VLC fraction	HPLC fraction[a]	method used for separation	solvent system[b]
1	1	1.5	NP[c]-HPLC	hexane
2	4 and 5		recrystallization	hexane
3	2	2.6	RP[d]-HPLC	acetone:H_2O (4:1)
4	2	2.5	RP[e]-HPLC	acetone:H_2O (7:3)
5	2	2.7	RP-HPLC	acetone:H_2O (4:1)
6	2	2.3.6	RP-HPLC	acetone:H_2O (4:1)
			NP-HPLC	hexane:TBME (49:1)
7	2	2.3.7	RP-HPLC	acetone:H_2O (4:1)
			NP-HPLC	hexane:CH_2Cl_2[f] (7:3)
8	2	2.3.6	RP-HPLC	acetone:H_2O (4:1)
			NP-HPLC	hexane:TBME (49:1)
9	2	2.4	RP-HPLC	acetone:H_2O (4:1)
10	2	2.4	RP-HPLC	acetone:H_2O (4:1)
11	2	2.7	RP-HPLC	acetone:H_2O (4:1)
12	2	2.4	RP-HPLC	acetone:H_2O (4:1)
13	2	2.3.3.1	RP-HPLC	acetone:H_2O (4:1)
			NP-HPLC	hexane:CH_2Cl_2[f] (7:3)
14	2	2.2	RP[e]-HPLC	acetone:H_2O (7:3)
15	2	2.3.3.1	RP-HPLC	acetone:H_2O (4:1)
			NP-HPLC	hexane:CH_2Cl_2[f] (7:3)

[a] An entry in this column indicates the sample was re-HPLCd at least once. The number of digits indicates the number of purification steps, e.g., 2.3.6 indicates VLC fraction 2, followed by two HPLC separations, where the further separated fractions were 3 from the first separation and then 6 from that separation. [b] All solvents were HPLC grade. [c] NP = normal phase silica. [d] RP = reversed phase silica (C_{18}) unless otherwise stated. [e] RP = reversed phase silica (C_2). [f] Water saturated.

Table 2. Physical and Spectroscopic Data for Compounds 1−15

compd	mass, mg (%)	$[\alpha]^{25}_D$, deg (*c*)[a]	mp, °C[b]	UV λ_{max} (ϵ)[c]	IR ν_{max}[d]	mol. formula	HREIMS (Δ mmu)	EIMS (%, RA)
1	2.6 (0.0019)	+45.0 (0.26)	oil	NR	2930, 2075, 1450, 1380	$C_{21}H_{33}N_S$	331.2344 (+2.8)	331 (12), 273 (6), 272 (9), 246 (3), 187 (20), 161 (30)
2	463.0 (0.33)	+43.8 (0.64)	107.5−108.5 cf. 109.0−110.0[4]	−	AP[3,4]	$C_{22}H_{32}N_2$	AP[3,4]	AP[3,4]
3	1.5 (0.0011)	+23.3 (0.15)	oil	247 (1250)	2930, 2130, 2120, 1460, 1380	$C_{22}H_{32}N_{2S}$	329.2143 (−1.7),[e] 297.2467 (+0.8)	329 (3), 297 (7), 271 (75), 270 (50), 255 (45)
4	7.5 (0.0055)	+36.1 (0.75)	oil	224 (1060)	3440, 2250, 2130, 1455, 1380	$C_{22}H_{32}N_{2O}$	340.2497 (±0.0)	340 (3), 298 (8), 297 (5), 270 (100), 255 (65), 217 (35)
5	5.8 (0.0042)	+37.0 (0.58)	oil	226 (1530)	2930, 2260, 2130, 1450, 1380	$C_{22}H_{32}N_2O$	340.2497 (±0.0)	340 (8), 325 (13), 313 (40), 298 (25), 270 (100), 255 (90)
6	10.7 (0.0076)	+4.9 (0.53)	124.3−125.6	−	2930, 2130, 1450, 1380	$C_{21}H_{30}N$	297.2467 (+0.9)	297 (20), 282 (30), 270 (80), 255 (100), 201 (45), 145 (50)
7	10.5 (0.0078)	+80.4 (0.53)	134.4−135.3	−	2940, 2130, 1460, 1380	$C_{21}H_{30}N$	297.2448 (−1.0)	297 (20), 282 (15), 270 (100), 255 (90), 214 (50), 199 (35)
8	75.0 (0.055)	+17.0 (1.89)	115.0−116.0	−	2935, 2130, 1455, 1380	$C_{21}H_{30}N$	297.2463 (+0.5)	297 (20), 282 (100), 270 (40), 255 (90), 199 (15)
9	3.8 (0.0028)	−3.7 (0.38)	oil	−	2920, 2130, 1450, 1380	$C_{21}H_{30}N$	297.2470 (+1.2)	297 (13), 282 (10), 270 (30), 186 (30), 159 (100)
10	16.9 (0.0123)	+14.0 (0.84)	148.0−149.0	−	2920, 2130, 1450, 1375	$C_{21}H_{30}N$	297.2421 (−3.7)	297 (25), 282 (60), 270 (100), 255 (80), 24, (45), 199 (55)
11	9.1 (0.0066)	+1.5 (0.55)	102.8−103.7	247 (1520)	2980, 2130, 2120, 1450, 1385	$C_{22}H_{32}N_2S$	356.2323 (−5.4)	356 (40), 329 (35), 270 (40), 255 (45), 201 (50), 159 (60)
12	36.8 (0.027)	+115.8 (1.23) cf. +116.8[5]	114.7−115.8 cf. 113.0−115.0[5]	−	2930, 2130, 1450, 1380	$C_{21}H_{30}N$	297.2482 (+2.4)	297 (8), 282 (35), 270 (60), 255 (70), 227 (60), 201 (100)
13	27.3 (0.0198)	−55.9 (0.90)	106.6−108.3	−	2925, 2130, 1450, 1380	$C_{21}H_{30}N$	297.2448 (−1.0)	297 (50), 282 (100), 270 (85), 255 (80), 199 (65), 159 (55)
14	5.6 (0.0041)	+78.4 (0.56)	oil	244 (1530)	3450, 2930, 2090, 1450, 1380	$C_{21}H_{30}NOS$	345.2131 (+0.3)	346 (50), 313 (20), 288 (25), 270 (100), 217 (60), 199 (40)
15	13.8 (0.01)	+67.0 (0.79)	oil	230 (7900)	2940, 2130, 1460, 1380	$C_{21}H_{30}N$	297.2442 (−1.6)	297 (20), 282 (65), 270 (40), 255 (65), 187 (70), 162 (65)

[a] In $CHCl_3$. [b] All were recrystallized from hexane. [c] In CH_3OH, nm. [d] As neat films, cm^{-1}. [e] Parent ion not observed, AP = as previously reported, − = not applicable, NR = not recorded.

parison with those for **2** and **3**, it was clear that **4** differed from both of these compounds by the substituent at C-7. In the case of **4** this substituent was deduced to be, on the basis of IR, UV, EIMS, and ^{13}C NMR spectral data (see Tables 2 and 3), an isocyanate function. As was the case for **3**, the stereochemical assignments made for **4**, based on ^{13}C NMR data and optical rotation comparisons with both **2** and **3** (see Tables 2 and 3), indicated it to be *iso*-structural with both of these compounds and to have the absolute configuration as shown. Compound **4** is (1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-20-isocyano-7-isocyanatoisocycloamphilectane.

Table 3. ^{13}C NMR (75.5 MHz, $CDCl_3$) Data for Compounds 1−15

carbon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	26.2 t[a,d]	40.5 d	40.7 d	40.8 d	40.9 d	42.1 d	38.3 d	36.9 d	41.5 d	34.0 d	33.2 d	34.4 d	35.7 d	42.4 d	136.9 d
2	35.6 t	42.0 t	42.1 t	42.3 t	42.4 t	41.9 t	43.6 t	42.4 t	44.1 t	40.9 t	41.8 t	42.4 t	41.0 t	37.3 t	48.8 t
3	30.8 d[c]	36.3 d	36.4 d	36.5 d	36.5 d	37.6 d	37.7 d	37.4 d	37.2 d	35.6 d	35.3 d	31.7 d	31.1 d	32.1 d	37.3 d
4	44.5 d	47.5 d	47.7 d	47.9 d	47.7 d	48.2 d	47.7 d	48.0 d	46.8 d	49.3 d	49.2 d	49.6 d	49.3 d	42.1 d	54.2 d
5	20.9 t	25.6 t	26.1 t	26.6 t	25.7 t	26.0 t	25.3 t	25.8 t	25.2 t	25.5 t	25.4 t	25.7 t	26.8 t	26.2 t	26.6 t
6	40.8 t	40.3 t	40.6 t	42.2 t	40.5 t	40.6 t	40.5 t	40.5 t	40.1 t	40.8 t	40.8 t	40.8 t	40.7 t	40.5 t	42.0 t
7	64.7 s[b]	60.3 br	64.4 s	61.2 br	60.5 br	60.6 br	59.8 br	60.5 br	60.2 br	60.6 br	60.5 br	60.8 br	61.1 br	65.0 s	61.3 s
8	48.8 d	48.1 d	48.9 d	49.7 d	48.4 d	49.1 d	44.6 d	48.8 d	44.9 d	49.9 d	49.9 d	49.8 d	48.6 d	44.7 d	47.2 d
9	24.1 t	25.6 t	26.1 t	25.9 t	25.4 t	26.0 t	25.5 t	26.6 t	26.0 t	30.2 t	30.3 t	27.3 t	22.9 t	27.6 t	20.1 t
10	30.6 t	25.1 t	25.1 t	25.3 t	25.8 t	28.5 t	118.5 d	33.3 t	122.3 d	37.2 t	37.3 t	35.9 t	32.6 t	32.0 t	28.0 t
11	135.2 s	48.5 d	48.7 d	48.8 d	50.1 d	40.2 d	136.5 s	134.5 s	136.7 s	150.7 s	150.8 s	148.6 s	125.9 s	150.1 s	35.7 d
12	121.3 d	45.7 d	45.9 d	46.0 d	46.4 d	42.5 d	48.7 d	48.6 d	49.9 d	51.3 d	51.4 d	48.2 d	132.4 s	74.1 s	46.1 s
13	38.1 d	45.1 d	45.8 d	46.2 d	45.4 d	45.6 d	43.5 d	46.3 d	44.3 d	48.3 d	48.0 d	40.9 d	40.1 d	43.9 d	53.0 d
14	124.6 d	37.6 t	37.7 t	37.8 t	38.2 t	125.5 d	46.3 t	44.3 t	133.2 d	43.1 t	46.8 t	126.4 d	127.0 d	127.1 d	129.9 d
15	131.4 s	39.8 d	39.9 d	40.0 d	41.3 d	138.2 s	31.6 s	31.5 s	126.8 s	144.4 s	60.4 s	130.3 s	129.6 s	129.8 s	142.2 s
16	25.7 q[e]	23.8 q	23.9 q	23.9 q	25.1 q	14.5 q	25.0 q	29.3 q	25.7 q	111.1 t	28.9q	26.0 q	26.1 q	26.0 q	114.7 t
17	17.7 q	16.0 q	16.1 q	16.1 q	16.3 q	22.3 q	32.3 q	31.4 q	17.5 q	22.6 q	32.1 q	17.7 q	17.7 q	17.8 q	18.8 q
18	13.2 q	19.5 q	19.6 q	19.7 q	19.7 q	19.7 q	19.5 q	19.7 q	19.5 q	19.7 q	19.8 q	20.0 q	19.8 q	19.9 q	16.8 q
19	20.0 q	20.3 q	20.4 q	21.6 q	20.4 q	20.3 q	20.9 q	20.4 q	21.0 q	20.7 q	20.7 q	20.5 q	20.2 q	20.5 q	20.3 q
20	23.7 q	64.9 br	65.1 br	65.1 br	64.5 br	38.1 d	47.6 t	131.1 d	24.9 q	105.6 t	105.8 t	108.1 t	19.2 q	110.7 t	15.7 q
21	129.7 br[f]	152.5 br	130.0 br	122.4 s	152.4 br	151.9 br	152.2 br	152.5 br	152.3 br	152.4 br	152.5 br	152.3 br	152.1 br	129.6 br	152.1 br
22		155.3 br	155.3 br	155.2 br	121.9 s						136.1 br				

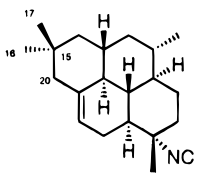
[a] Multiplicity by DEPT. [b] s = C. [c] d = CH. [d] t = CH_2. [e] q = CH_3. [f] br = broad singlet or a poorly resolved triplet.

Compound 5. EIMS analysis of **5** showed it to have the same molecular formula as **4** ($C_{22}H_{32}N_2O$). The UV and IR data for **4** and **5** were also comparable, indicating them to have identical functionality. From the ^1H and ^{13}C NMR data of **5** (see Tables 3 and 4), it was also evident that the only differences between **5** and **4** were a result of the −CN and −NCO funtionalities switching positions. As was the case for the three previous compounds, the stereochemical assignments made for **5**, based on ^{13}C NMR data and optical rotation comparisons with **2**, **3**, and **4** (see Tables 2 and 3), indicated it to be *iso*-structural with all of these compounds and to have the absolute configuration as shown in **5**. Compound **5** is (1*S*,3*S*,4*R*,7*S*,8*S*,11*S*,12*S*,13*S*,15*R*,20*R*)-20-isocyanto-7-isocyanoisocycloamphilectane.

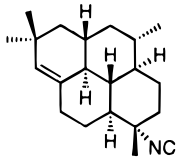
Compounds **4** and **5** are not only the first representatives of isocycloamphilectane-based compounds to contain both isonitrile and isocyanate functionalities within the same molecule but also the only known marine natural products to do so. They are also only the second and third examples of marine-derived natural products known to contain an isocyanate function, the other also being derived from a marine sponge.[8]

Compound 6. The accurate molecular mass of **6** indicated it to have the molecular formula $C_{21}H_{30}N$. From its IR and ^1H and ^{13}C NMR data (see Tables 3 and 4) it appeared that **6** was also an isocycloamphilectane derivative containing a single carbon−carbon double bond (125.5 (d), 138.2 (s) ppm) and an isonitrile function. On the basis of long-range ^1H and ^{13}C 2D NMR correlations observed between H_3-19 and C-6, C-7 and C-8 the isonitrile was positioned at C-7, as in **1** and **5**. The $\Delta^{14,15}$ double bond was positioned also on the basis of long-range ^1H and ^{13}C 2D NMR correlations, this time between H_3-16 and C-11, C-15, and C-20 and between H_3-17 and C-14, C-15, and C-20, with H-14 also showing correlations to C-1, C-15, C-17, and C-20. The stereochemistry shown for **6** was proposed on the basis of ^1H and ^{13}C NMR data (see Tables 3 and 4), comparisons made between **6** and **2**−**5**, and upon NOESY results obtained for **6**. Unfortunately these data were not adequate for the assignment of C-20, which was eventually assigned from the results of a single-crystal X-ray crystallographic analysis.[10] As the sign of the optical rotation and the ^1H and ^{13}C NMR data for **6** are so similar to those for compounds **2**−**5**, it is proposed that the view shown of **6** represents its absolute relative configuration. Compound **6** is thus (1*S**,3*S**,4*R**,7*S*,8*S**,11*R**,12*R**,13*S**,20*S**)-7-isocyanoisocycloamphilect-14-ene.

Cycloamphilectane-Based Isolates (7 and 8). Compound 7. The mass spectral data for **7** indicated that it has the identical molecular formula to that found for **6**, $C_{21}H_{30}N$. It was also apparent from these data and its IR and ^1H and ^{13}C NMR data (see Tables 3 and 4) that it too was tetracyclic and contained a single carbon−carbon double bond (118.5 (d), 136.5 (s) ppm) and an isonitrile function. The HMBC data, in contrast to those for **6**, clearly showed **7** to contain a *gem*-dimethyl moiety at C-15, indicating the molecule to be cycloamphilectane based. The double bond was assigned as being $\Delta^{10,11}$ on the basis of long-range ^1H and ^{13}C NMR correlations observed between H-10 and C-8, C-9, and C-20, as well as the $^1H^1H$ couplings observed between H-10 and H_2-9 and between H_2-9 and H-8. Stereochemically **7** was shown to have the same relative configurations at comparable stereocenters to those found in all of the previously discussed molecules on the basis of ^1H and ^{13}C NMR data comparisons and NOESY measurements made with **7**. Compound **7** is (1*S**,3*S**,4*R**,7*S**,8*S**,-12*S**,13*S**)-7-isocyanocycloamphilect-10-ene.



7



8

Compound 8. In most respects the spectroscopic data for **8** revealed it, with the exception of the position of its carbon−carbon double bond, to be identical to **7**. From long-range ^1H and ^{13}C NMR correlations observed between H_3-16/H_3-17 and C-14, C-15, and C-20 in the HMBC spectrum of **8**, it was possible to assign the carbon−carbon double bond as being $\Delta^{11,20}$. Considering that **8** was crystalline and the fact that our deductions concerning its relative configuration were not conclusive,

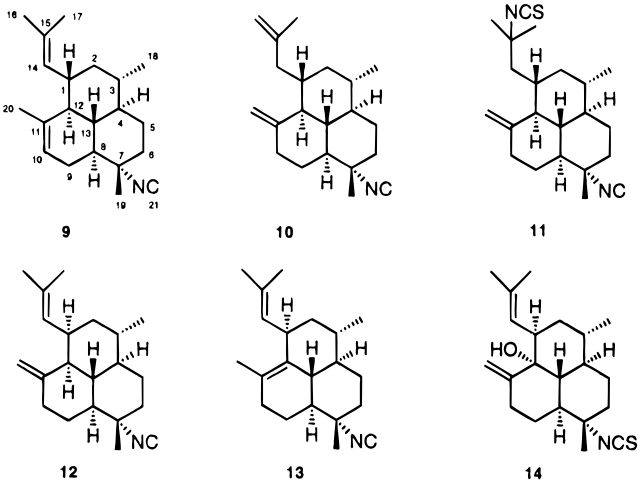
Table 4. ^{1}H NMR (300 MHz, $CDCl_3$) Data for Compounds 1 and 3−15[a]

proton	1	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.93 (m)	1.97 (m)	1.13 (m)	1.96 (m)	1.73 (m)	1.29 (m)	1.41 (m)	2.22 (m)	1.65 (m)	1.87 (m)	2.88 (dm, J = 8.7)	3.62 (d, J = 2.8, 5.0, 9.7)	2.70 (m)	5.65 (d, J = 16.1)
2	1.24 (m)	0.83 (m), 1.54 (ddd, J = 3.5, 3.5, 12.9)	0.84 (m), 1.54 (m)	0.81 (m), 1.55 (dddd, J = 3.2, 3.2, 3.2, 9.4)	0.84 (m), 1.66 (m)	0.86 (m), 1.59 (m)	0.89 (m), 1.59 (ddd, J = 3.1, 3.8, 12.6)	0.92 (m), 1.53 (m)	0.66 (m), 1.82 (m)	0.86 (m), 2.21 (m)	1.26 (m), 1.62 (dd, J = 2.9, 9.0)	1.21 (m), 1.51 (ddd, J = 2.8, 3.1, 12.3)	1.38 (m), 1.84 (m)	1.06 (m), 2.14 (dd, J = 5.4, 12.9)
3	1.93 (m)	1.13 (m)	1.12 (m)	1.12 (m)	1.20 (m)	1.22 (m)	1.23 (m)	1.21 (m)	1.04 (m)	1.09 (m)	1.26 (m)	1.36 (m)	1.23 (m)	1.44 (m)
4	1.15 (m)	0.74 (m)	0.74 (m)	0.74 (m)	0.78 (m)	0.81 (m)	1.32 (m)	0.76 (m)	0.81 (m)	0.84 (m)	0.79 (m)	0.69 (dddd, J = 3.5, 10.9, 11.1, 11.1)	1.31 (m)	0.97 (m)
5	1.14 (m), 1.53 (m)	0.93 (m), 1.77 (ddd, J = 3.9, 13.0,13.0)	0.92 (m), 1.87 (m)	0.91 (m), 1.84 (dddd, J = 3.7, 3.7, 3.9, 13.6)	0.93 (m), 1.88 (dddd, J = 3.3, 3.6, 3.8, 13.1)	0.98 (m), 1.84 (m)	0.93 (m), 1.88 (m)	0.95 (m), 1.88 (m)	0.90 (m), 1.88 (m)	0.94 (m), 1.87 (m)	0.96 (m), 1.87 (m)	1.02 (m), 1.93 (m)	0.91 (m), 1.94 (m)	1.14 (m), 1.84 (m)
6	1.77 (m), 2.01 (m)	1.90 (dddd, J = 3.2, 3.2, 3.9, 13.5), 1.98 (m)	1.68 (m), 1.91 (m)	1.81 (ddd, J = 3.9, 13.0, 13.1), 2.01 (m)	1.81 (ddd, J = 4.0, 13.1, 13.1), 2.02 (m)	1.81 (m), 2.03 (dm, J = 14.0)	1.82 (ddd, J = 4.0, 13.0, 13.1), 2.04 (m)	1.84 (m), 2.02 (m)	1.85 (m), 2.04 (dm, J = 13.9)	1.83 (m), 2.03 (m)	1.87 (m), 2.06 (m)	1.86 (ddd, J = 4.3, 13.0, 13.1), 2.07 (m)	1.80 (m), 1.97 (m)	1.83 (m), 2.09 (dm, J = 15.4)
8	1.50 (ddd, J = 1.7, 10.3, 10.3)	1.41 (m)	1.26 (m)	1.38 (m)	1.36 (m)	1.71 (m)	1.43 (m)	1.74 (m)	1.63 (m)	1.62 (m)	1.53 (ddd, J = 3.0, 11.6, 11.6)	1.56 (ddd, J = 2.4, 12.5, 12.5)	1.99 (m)	1.77 (m)
9	1.36 (m), 1.93 (m)	1.12 (m), 2.03 (m)	1.06 (m), 2.01 (m)	1.07 (m), 2.11 (dddd, J = 2.5, 2.5, 2.8, 11.7)	1.07 (dddd, J = 3.2, 12.2, 12.2, 12.2), 2.03 (m)	1.93 (m), 2.25 (dm, J = 16.5)	1.07 (m), 1.98 (m)	1.72 (m), 2.28 (m)	1.25 (m), 2.21 (dm, J = 12.4)	1.26 (m), 2.21 (m)	1.16 (m), 2.09 (m)	1.28 (m), 1.94 (m)	1.17 (m), 1.94 (m)	1.32 (m), 1.81 (m)
10	1.93 (m), 2.01 (m)	1.32 (m), 2.04 (m)	1.33 (m), 2.00 (m)	1.25 (m), 2.05 (m)	1.32 (m), 1.63 (m)	5.33 (br d, J = 6.0)	2.01 (m), 2.26 (dm, J = 14.1)	5.40 (m)	1.96 (br ddd, J = 4.3, 8.6, 8.7), 2.40 (ddd, J = 2.8, 4.3, 12.4)	1.93 (m), 2.40 (ddd, J = 2.8, 4.4, 12.2)	2.06 (m), 2.38 (m)	1.94 (m), 2.15 (m)	2.19 (ddd, J = 3.2, 3.7, 13.6), 2.54 (br t, J = 13.6)	1.32 (m), 1.69 (m)
11	–	0.95 (m)	0.94 (m)	0.97 (m)	1.44 (m)	–	–	–	–	–	–	–	–	1.57 (m)
12	5.34 (br s)	0.94 (m)	0.93 (m)	0.92 (m)	0.81 (m)	1.32 (m)	0.81 (m)	1.73 (m)	1.45 (m)	1.44 (m)	1.87 (m)	–	–	–
13	1.77 (m)	0.69 (m)	0.67 (m)	0.64 (m)	0.69 (m)	0.88 (m)	0.65 (ddd, J = 10.0, 10.0, 11.0)	0.96 (m)	0.78 (m)	0.81 (m)	1.17 (m)	1.75 (m)	1.32 (m)	1.13 (m)
14	5.10 (br t, J = 7.2)	1.15 (m), 1.44 (m)	1.13 (m), 1.42 (m)	1.09 (m), 1.37 (m)	5.08 (br s)	0.88 (m), 1.83 (m)	1.08 (m), 1.32 (m)	5.07 (br d, J = 9.1)	1.48 (br d, J = 14.1), 2.63 (dd, J = 10.3, 14.1)	1.22 (m), 2.10 (dd, J = 1.7, 14.6)	5.23 (br d, J = 8.9)	5.26 (br d, J = 9.7)	5.19 (br d, J = 8.9)	6.10 (d, J = 16.1)
15	–	1.45 (m)	1.45 (m)	1.46 (m)	–	–	–	–	–	–	–	–	–	–
16	1.69 (br s)	1.36 (t, J = 1.8)	1.36 (t, J = 1.9)	1.32 (m)	0.89 (d, J = 7.4)	0.77 (s)	0.95 (br s)	1.65 (br s)	4.65 (br s), 4.74 (br s)	1.43 (s)	1.70 (br s)	1.68 (d, J = 1.4)	1.64 (s)	4.90 (br s)
17	1.60 (br s)	1.02 (d, J = 6.4)	1.02 (d, J = 6.8)	0.96 (d, J = 6.7)	1.67 (br s)	0.91 (s)	0.93 (br s)	1.56 (br s)	1.72 (s)	1.45 (s)	1.65 (br s)	1.67 (d, J = 1.4)	1.70 (m)	1.83 (br s)
18	0.75 (d, J = 6.9)	0.86 (d, J = 6.5)	0.87 (d, J = 6.7)	0.87 (d, J = 6.5)	0.89 (d, J = 6.5)	0.89 (d, J = 6.5)	0.90 (d, J = 6.5)	0.85 (d, J = 6.4)	0.86 (d, J = 6.7)	0.90 (d, J = 6.6)	0.83	0.83 (d, J = 6.4)	0.84 (d, J = 6.1)	0.93 (d, J = 6.4)
19	1.29 (s)	1.27 (s)	1.20 (s)	1.28 (t, J = 2.1)	1.29 (br s)	1.31 (br s)	1.27 (br s)	1.30 (br s)	1.28 (br s)	1.28 (t, J = 1.9)	1.32 (br s)	1.34 (br s)	1.31 (s)	1.32 (br s)
20	1.68 (br s)	–	–	–	1.96 (m)	0.96 (m), 1.28 (m)	5.05 (br s)	1.65 (br s)	4.60 (br s), 4.84 (br s)	4.53 (br s), 4.85 (br s)	4.52 (ddd, J = 1.8, 1.8, 1.8), 4.72 (ddd, J = 1.8, 1.8, 1.8)	1.62 (br s)	4.80 (br s)	0.79 (d, J = 6.4)

[a] All assignments are based on extensive 1D and 2D NMR experiments, including COSY90, HMQC, HMBC, HETCOR, and NOESY. [b] J values are in hertz. [c] – = not applicable.

a single-crystal X-ray analysis was made,[10] the results of which were consistent with compound **8** being named as (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanocycloamphilect-11(20)-ene. These findings also supported the deductions made concerning the stereochemical assignments proposed for **7**.

Amphilectane-Based Isolates (9−14). Compound 9. The molecular mass of **9** indicated it to have the molecular formula $C_{21}H_{30}N$. Of the seven degrees of unsaturation indicated by the molecular formula of **9**, four were occupied in multiple bonds, two carbon−carbon double bonds (122.3 (d), 126.8 (s), 133.2 (d), 136.7 (s) ppm) and a single CN triple bond (152.3 (br) ppm, 2130 cm^{-1}), indicating the molecule to be tricyclic. After the association of all of its ^1H and ^{13}C NMR resonances (see Tables 3 and 4), it was possible to deduce a number of molecular fragments from its $^1H-^1H$ COSY and ^1H and ^{13}C NMR data and comparisons with those of compounds **2**−**8**. Thus, it was evident from direct data comparisons that the C-1 through C-13 portion of **9** was essentially identical to the comparable parts of **2**−**8**, clearly indicating the fourth ring of these systems not to be intact. From $^1H-^1H$ coupling information it was also evident that a 2-methylpropenyl moiety was located at C-1. Thus, both H_3-16 and H_3-17 (δ 1.56 (br s), 1.65 (br s)) demonstrated allylic coupling to H-14 (δ 5.07 (br d, *J* 9.1 Hz)), which, in turn, coupled to H-1 (δ 2.22 (m)). In turn H-1 coupled to both H-12 and H_2-2. Both H-10 and H-14 showed long-range ^1H and ^{13}C coupling to H-12, as did H_3-20, thus confirming **9** to have the ground structure of a C-7 isonitrile, $\Delta^{14,15}$,$\Delta^{10,11}$-substituted amphilectane. The relative configurations for stereocenters C-3, C-4, C-7, C-8, C-12, and C-13 were deduced to be identical to the equivalent ones found in **2**−**8** and especially **7** on the basis of ^{13}C NMR data comparisons made between these sets of data. From the NOESY spectrum of **9** it was also clear, by the presence of cross peaks between H-13 and H_3-19, and H-13 and H-1, that they must be β and axial and the 2-methylpropenyl moiety must be α and equatorial. Compound **9** is thus (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-10,14-diene.



Compound 10. Mass spectral analysis indicated **10** to have the same molecular formula as **9**. Detailed analysis of its other spectroscopic data, IR and ^1H and ^{13}C NMR, also revealed it to be an amphilectane-based diterpene having an isonitrile group at C-7, and two terminal alkenyl carbon−carbon double bonds, $\Delta^{11,20}$ and $\Delta^{15,16}$. A literature search based on this information indicated that **10** was probably identical with the compound of Kazlauskas *et al.*,[5] called 7-isocyano-11(20),15-epiamphilectadiene. However, the general lack of IR and ^{13}C NMR spectra in this work,[5] as well as the absence of optical rotation and melting point, made this deduction at best speculative. After all of the NMR data for **10** had been fully assigned (see Tables 3 and 4), it was decided that, since suitable crystals were available, a single-crystal X-ray analysis should be made so as to establish the relative configuration of this molecule unequivocally. The results of this analysis[10] were consistent with compound **10** being named as (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-11(20),15-diene.

Compound 11. The spectroscopic data for **11** indicated it to be a molecule very similar to **10**, but lacking the $\Delta^{15,16}$ double bond and having in its place a tertiary methyl group (δ 1.43 (s), 28.9 (q) ppm) and an isothiocyanate function (247 nm, 2120 cm^{-1}, 60.4 (s), 136.1 (br) ppm). In all other respects the two molecules were identical. Compound **11** is thus (1*S**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyano-15-isothiocyanatoamphilect-11(20)-ene.

Compound 12. After detailed spectroscopic analysis, **12** was concluded to be the known compound 7-isocyano-11(20),14-epiamphilectadiene isolated previously by Kazlauskas *et al.*,[5] for which the new semisystematic name (1*R**,3*S**,4*R**,7*S**,8*S**,12*S**,13*S**)-7-isocyanoamphilecta-11(20),14-diene is proposed. On the basis of extensive 1D and 2D NMR measurements made with this compound, we are able to report complete and unambiguous data for this compound for the first time (see Tables 3 and 4). It is noteworthy that the C_4 side chain at C-1 in **12** is β and hence on the opposite side of the molecule to that found for the equivalent grouping in compounds **9**−**11**.

Compound 13. The isonitrile **13** was clearly the $\Delta^{11,12}$ double-bond isomer of **12**, on the basis of the presence of ^1H and ^{13}C NMR resonances for a tetrasubstituted carbon−carbon double bond (125.9 (s), 132.4 (s) ppm) and an olefinic methyl group (δ 1.62 (br s), 19.2 (q) ppm) in **13**, together with the absence of ^1H and ^{13}C NMR resonances associated with the *exo*-methylene group found in **12**. In all other respects **12** and **13** appeared identical. Stereochemically, **12** and **13** were also concluded to be identical, in a relative sense, at all corresponding chiral centers on the basis of ^1H and ^{13}C NMR data comparisons and NOESY measurements made with **13**. The configuration at C-1 was concluded to be as shown in **13**, on the basis of cross peaks observed in the NOESY spectrum between H_3-19 and H-13 and between H-13 and H-14, indicating them to be axial and β. Compound **13** is thus (1*R**,3*S**,4*R**,7*S**,8*S**,13*R**)-7-isocyanoamphilecta-11,14-diene.

Compound 14. By mass spectrometry, **14** analyzed for $C_{21}H_{30}NOS$. From the UV, IR, and ^{13}C NMR data of **14**, it was evident that the functionality within the molecule was present in the form of a tertiary alcohol (3450 cm^{-1}, 74.1 (s) ppm) and a tertiary isothiocyanate moiety (244 nm, 2090 cm^{-1}, 65.0 (s), 129.6 (s) ppm). The ^{13}C NMR data also indicated the presence of two carbon−carbon double bonds as the only other multiple bonds present in the molecule; **14** is thus tricyclic. From these data it was also clear that **14** had the same planar

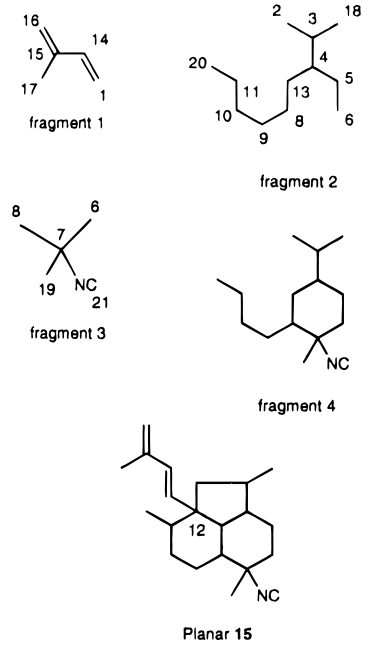
(10) Linden, A.; König, G. K.; Wright, A. D. *Acta Crystallogr., Sect.* C, in press.

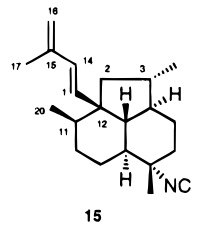
structure as **12**. The isothiocyanate function was positioned at C-7 on the basis of both long-range $^1H-^{13}C$ correlations observed between H_3-19 and C-7 and ^{13}C NMR comparisons made between its data and those for **1** and **3**. The OH group was clearly located at C-12, since H_2-20, H-14, H_2-10, H_2-2, and H-1 all showed $^1H-^{13}C$ correlations to C-12. On the basis of an NOE interaction between H-14 and H-13, it seemed reasonable to conclude that the 2-methylpropenyl moiety and H-13 were axial and β as is H_3-19. It also seemed likely from the spectroscopic data that centers C-3, C-4 and C-8 also had the same relative configurations to those found in all of the previous molecules. The configuration at C-12, however, was not so easily resolved, since all of the available data suggested either an α or a β orientation of the OH group was possible. In an attempt to resolve this impasse unequivocally, a shift reagent study was undertaken.[11,12] The results of this study, while confirming all of the previous findings, did not allow an unambiguous assignment of configuration to C-12. As a result of these findings we were forced to go back and review all of our NMR data and make more careful comparisons with all of the available data. What became apparent from this was that all of the resonances for carbons in a 1,3 relationship with C-12 (see highlighted resonances in Table 3) were considerably shielded when compared with equivalent centers in all of the other isolates. This observation is consistent with recent findings by Nick *et al.*,[13,14] which clearly indicate that in such circumstances the OH group must be axial and, in the case of **14**, α also. Compound **14** is thus (1*R**,3*S**,4*R**,7*S**,8*S**,12*R**,13*R**)-12-hydroxy-7-isothiocyanatoamphilecta-11(20),14-diene.

Compound **14** is the first example of the amphilectane/cycloamphilectane series of compounds to contain an oxygen-based functional group, and in this respect it is an unprecedented natural product.

Isoamphilectane-Based Isolates. Compound 15. The molecular mass of **15** indicated it to have the molecular formula $C_{21}H_{30}N$. The ^{13}C NMR data of **15** showed the presence of three multiple bonds: two carbon−carbon double bonds (122.3 (d), 126.8 (s), 133.2 (d), 136.7 (s) ppm) and a single CN triple bond (152.3 (br) ppm, 2130 cm^{-1}), indicating the molecule to be tricyclic. After the association of all of the ^1H and ^{13}C NMR resonances (see Tables 3 and 4), it was possible to deduce a number of molecular fragments from its $^1H-^1H$ COSY. Thus, cross peaks observed between H_2-16 and H_3-17, H-14, and H-1 and between H-1 and H-14, combined with the facts that the two carbon−carbon double bonds within **15** must be conjugated (UV, 230 nm (ϵ 7900)) and that $J_{1,14}$ = 16.1 Hz, clearly delineated fragment 1, Scheme 2. Further, as evidenced by the cross peaks in the $^1H-^1H$ COSY of **15**, H_2-2 coupled H-3, which in turn coupled to H_3-18 and H-4. H-4 further coupled to H_2-5 and to H-13. H_2-5 in turn coupled to H_2-6, which showed no further coupling. H-13 further coupled to H-8, which demonstrated coupling to H_2-9. These latter two protons intercoupled and further coupled to H_2-10, which coupled to H-11, which showed further coupling only to H_3-20, thus completing fragment 2, Scheme 2. The third molecular fragment shown in Scheme 2 was deduced from long-range $^1H-^{13}C$ correlations observed between H_3-19 and C-6, C-7, and C-8, clearly enabling it to be incorporated into fragment 2 and so to generate fragment 4. This now leaves a fully substituted carbon atom (C-12) and fragments 1 and 4 to be united to generate planar **15**, Scheme 2, as the only logical structure based on these data. The relative configurations at C-7 and C-8 in **15** are proposed to be the same as those of equivalent centers in all of the previously discussed molecules on the basis of comparable NMR data. The stereochemistry at all of the remaining chiral centers was proposed from the data contained in the NOESY spectrum of **15**. Thus, cross peaks observed between H-14 and H-3 and H_3-19 and between H-1/H-14 and H-13 and H_3-20 clearly position them all on the same face of the molecule (β) and dictate that H-4 and H-8 be on the opposite face (α). Compound **15** is thus the first member of a new class of tricyclic diterpenes for which the semisystematic name (1(14)-*E*,3*S**,4*R**,7*S**,8*S**,11*R**,12*R**,13*R**)-7-isocyanoneoamphilecta-1(14),15-diene is proposed.

Scheme 2





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Comment on Naming. As the contents of this publication more than doubles the current information concerning the three major classes of diterpenes discussed, we considered this an appropriate time to further rationalize the trivial/semisystematic naming of these compounds. Thus, to be consistent with the reports of Chang and Scheuer[15] and Wratten and Faulkner,[16] it is

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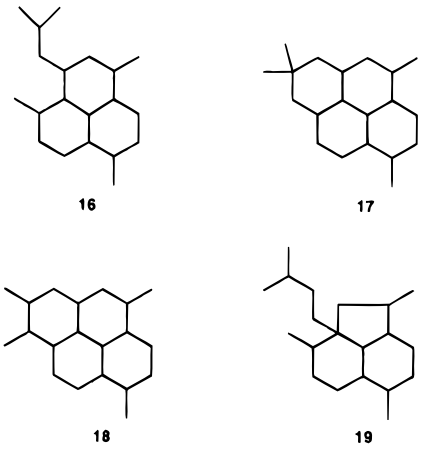
Table 5. Cytotoxicity of Compounds 1−15 to KB Cells and *P. falciparum* Clones D6 and W2 (Antimalarial Activity)

	KB cells	clone D6		clone W2	
compd	IC_{50} (ng/mL)	IC_{50} (ng/mL)	SI[a]	IC_{50} (ng/mL)	SI
1	>20 000	>10 000		>10 000	
2	4700	4.7	1000	4.3	1100
3	1600	45.1	35.5	28.5	56.1
4	2000	74.9	26.7	56.1	35.7
5	4300	3.2	1340	2.5	1710
6	18 200	62.5	290	19.5	930
7	>20 000	84.9	>240	28.4	>700
8	14 500	74.1	200	23.8	610
9	>20 000	302	>66.2	133	>150
10	>20 000	520	>38.5	242	>82.6
11	>20 000	470	>42.6	109	>183
12	3200	14.1	230	9.3	340
13	15 200	58.5	260	25.6	590
14	5300	797	6.6	423	12.5
15	19 100	90.0	210	29.7	640
		Antimalarial Standards			
chloroquine	17 400	3.8	4,600	50.5	340
quinine	>20 000	19.4	>1030	54.6	>370
mefloquine	3500	11.5	300	3.8	920
artemisinin	>20 000	2.8	>7140	2.1	>9400

[a] Selectivity index (SI) is defined as the ratio KB cell cytotoxicity over *P. falciparum* clones D6 or W2 cytotoxicity (antimalarial activity).

proposed that compounds based on hydrocarbon skeleton **16** be amphilectane derivatives, those based on hydrocarbon skeleton **17** be cycloamphilectane derivatives, and those based on hydrocarbon skeleton **18** be isocycloamphilectane derivatives, as has been done throughout the current report. For compound **15,** its trivial/semisystematic name is based on the hypothetical alkane nucleus **19**, for which the trivial/semisystematic name of neoamphilectane is proposed.



Biological Activity. All of the compounds reported here have been tested for biological activity *in vitro* against two clones of the malaria parasite *Plasmodium falciparum*. With the exception of **1**, all compounds demonstrated significant *in vitro* antiplasmodial activity (see Table 5). Further evaluation of the isolates against the mammalian KB cell line facilitated the calculation of an experimental selectivity index (SI) in order to assess whether the observed antiplasmodial activity was a specific or general toxic effect. Modification of the isonitrile functionality at C-7 in amphilectane skeleton **16** or **18** (i.e., compounds **3**, **4**, and **14**) results in markedly decreased antiplasmodial potency with little concomitant impact upon KB cytotoxicity; subsequently, the selectivity indices of these compounds are relatively low. In contrast, a number of the isolates exhibit several hundred times more inhibition of *Plasmodium* than of mammalian KB cells *in vitro* and are worthy of further study as potential antimalarial agents. Compounds **2** and **5**, in particular, display antiplasmodial potency and selectivity that rivals the *in vitro* results obtained with some clinically-used antimalarial drugs.

Summary and Conclusions. The information contained in this report more than doubles the information in the literature concerning the classes of diterpene isonitrile discussed, as well as providing the first comprehensive listing of fully reported and assigned $^{1}H-^{13}C$ NMR data for the same. On the basis of these data, researchers in the future should be able to assign structurally similar compounds much more easily than at present. It is also likely from the findings of this work that isonitrile, isocyanate, and isothiocyanate functionalities can be expected to be found within the same molecule, in sponge secondary metabolites, on a much more regular basis in the future. In addition, the potent and selective biological activities of these compounds represent an exciting advance in the search for novel antimalarial agents at a time when the efficacy of many of the currently available drugs is declining. Several of the compounds described here are clearly viable lead candidates for further development, and a detailed investigation into their structure−activity relationships is presently ongoing.

Experimental Section

General Experimental Procedures. Remaining details as per ref 17.

Materials. All sponge materials were collected by divers, using SCUBA, from Kelso Reef, Queensland, Australia. The animals were all collected from a depth of 9−12 m during March of 1993 and then deep frozen. A voucher specimen is deposited with Museum d'Histoire Naturelle, Geneva, Switzerland.

Extraction and Isolation. Deep frozen sponge tissue was freeze dried. Dry tissue (137.7 g) was extracted with dichloromethane (3 L) and then with acetone (3 L). From both

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(17) König, G. M.; Wright, A. D.; Sticher, O. *J. Nat. Prod.* **1990**, *53*, 1615.

extracts the dichloromethane solubles (5.2 g, 3.78%) were taken, combined, and chromatographed over silica (VLC, vacuum liquid chromatography) with petroleum ether containing increasing proportions of ethyl acetate as eluent; 11 fractions each of approximately 90 mL were obtained. TLC, ^{1}H NMR, and antimalarial screen results indicated VLC fractions 1−5 to be the most interesting. HPLC separation of VLC fractions 1 and 2 afforded two previously reported compounds (**12** and **10**)[5] and 12 new diterpene metabolites (**1**, **3**−**9**, **11** and **13**−**15**). VLC fractions 4 and 5 were pure **2** (diisocyanoadociane).[3,4,8]

For methods of separation see Table 1. For physical and spectroscopic data, see Tables 2−4.

Antiplasmodial Bioassay. Cultures of *P. falciparum* (chloroquine-sensitive clone D6 derived from CDC Sierra Leone and chloroquine-resistant clone W2 derived from CDC Indochina III) were maintained in human erythrocytes according to established methods.[18] Parasites were inoculated into type A+ human erythrocytes at a hematocrit of 6 % in RPMI-1640 culture medium (GIBCO Laboratories, Grand Island, NY) supplemented with 32 mmol of $NaHCO_3$ (GIBCO), 25 mmol of HEPES (*N*-(2-hydroxyethyl)piperazine-*N*-2-ethanesulfonic acid, Sigma Chemical Co., St. Louis, MO), and 10% heat-inactivated human plasma type A+. Parasitemia was maintained below 4% under an atmosphere of 5% O_2, 5% CO_2, and 90% N_2 in 25 cm^3 culture flasks at 37 °C.

The antiplasmodial activity of test compounds was assessed with an *in vitro* [^{3}H]hypoxanthine-incorporation method as previously detailed.[19,20] Concentrations of both test compounds and positive controls which inhibited parasite-specific incorporation of [^{3}H]hypoxanthine by 50% (IC_{50}) were determined by nonlinear regression analysis.

Cytotoxicity Bioassay. The KB-3 cell line was supplied by Dr. I. B. Roninson (Department of Genetics, University of Illinois College of Medicine at Chicago, Chicago, IL). KB-3 cells were cultured in D-MEM (GIBCO) supplemented with 10% fetal bovine serum (Atlanta Biologicals) and penicillin−streptomycin−fungizone (GIBCO) at 37 °C in 100% humidity with a 5% CO_2 atmosphere in air.

Evaluation of the cytotoxic potential of each of the test compounds was performed essentially as previously described,[19,20] by incubating mammalian KB cells with the test compound and quantifying cell growth spectrophotometrically with the protein-binding dye sulforhodamine B. The mean absorption values obtained with each of the treatment procedures were expressed as a percentage, relative to the solvent-treated control incubations, and IC_{50} values were calculated using nonlinear regression analysis (percent survival versus concentration).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra (15 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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