**Acknowledgment.** We thank Dr. Valerie Paul of the University of Guam for invaluable help in the biosynthetic experiments with *Acanthella*, and M. R. Hagadone and N. K. Gulavita for supplying the crude hydrocarbon mixture from *Ciocalypta*. Thanks also to A. Poiner and J. Hill for assistance with the tedious chromatography of **10**. We acknowledge financial support from the National Science Foundation and from the University of Hawaii Sea Grant College Program under Institutional Grant NA81AA-D-0070 from NOAA, Office of Sea Grant, U.S. Department of Commerce.

**Registry No. 8**, 73069-50-8; **9**, 893898-14-1; **10**, 119323-93-2; **11**, 93426-91-6; **11** (triamine deriv), 119244-33-6; **12**, 119244-32-5; $CN^-$, 57-12-5; benzyl isocyanate, 3173-56-6.

# Isocyanoneopupukeanane, a New Tricyclic Sesquiterpene from a Sponge[1]

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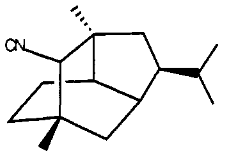
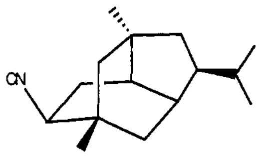
A sponge, *Ciocalypta* sp., from the south shore of O'ahu produces an isocyanosesquiterpene with a new tricyclic symmetrical skeleton. Its structure was determined by extensive NMR measurements, including long-range carbon–hydrogen, NOE, and COSY connectivities.

Our biosynthetic experiments toward discovering the origin of the isocyano function in marine sponges[2a,b] have been carried out on *Ciocalypta* sp. from the north shore of O'ahu, HI, where the animal is relatively abundant. More importantly, it contains one major metabolite, 2-isocyanopupukeanane (**1**).[3] High surf makes the north shore of O'ahu inaccessible to diving between October and April. We therefore searched south shore reefs for *Ciocalypta* sp. and found a few specimens on the Ala Moana reef. We injected potassium [$^{13}C^{15}N$]cyanide[2b] into a sponge, *Ciocalypta* sp., only to discover during the isolation that its principal secondary metabolite was not 2-isocyanopupukeanane (**1**) but a new and rearranged tricyclic isocyanosesquiterpene, the structure of which is the subject of this report.

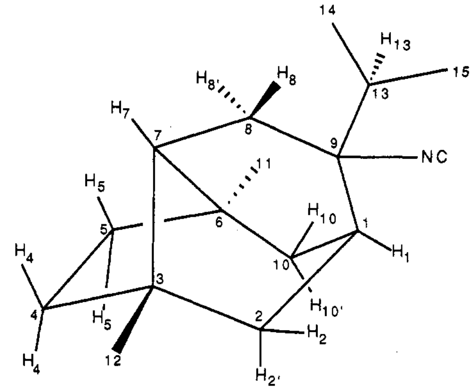
The frozen sponge was blended three times in ethanol. The filtrate was concentrated and partitioned against hexane. The residue of the hexane extract was chromatographed, yielding a complex mixture of which the major constituent was a colorless oil, rather than the anticipated low-melting solid. The highest peak in the EI mass spectrum of the new compound, isocyano*neo*pupukeanane, at *m/z* 231.1988 corresponded to a molecular formula of $C_{16}H_{25}N$, identical with the composition of **1**. Lack of olefinic $^{13}C$ or $^1H$ NMR signals indicated that the compound was a tricyclic saturated sesquiterpene. These compounds possess a few salient spectral features, but there was ample evidence that the compound bore an isocyano function ($\nu_{max}$ 2115 cm$^{-1}$; lack of UV absorption). Secondly, the compound clearly differed from **1** and from the isomeric 9-isocyanopupukeanane (**2**).[4] In contrast to **1** and **2**, in which the isocyano function is linked to a methine, the corresponding carbon (C-9, $\delta$ 64.5) in the new compound is quaternary and is only coupled to $^{14}N$ (t, $J \sim 4$ Hz). That the new compound furthermore must possess a new skeleton was seen by its five methylenes [$\delta$ 41.7 (2×), 39.9, 38.8, 32.1] in contrast to **1** and **2** with three methylenes each. Extensive NMR spectral measurements established the structure of 9-isocyano*neo*pupukeanane (3,6-dimethyl-9-isopropyltricyclo[4.3.1$0^{3,7}$]decane) (**3**).



**1**



**2**



**3**

The $^1H$ decoupled $^{13}C$ NMR spectrum (Table I) revealed 15 resonances with the signal at 41.7 ppm corresponding to two carbons. Changing solvents from benzene to chloroform, acetone, or cyclohexane did not separate these signals. The fully coupled DEPT spectrum[5] provided the multiplicity and $^1J_{CH}$ coupling constants for all carbons. The signals at 158.3 and 64.5 ppm are 1:1:1 triplets ($J \sim 4$ Hz), typical of carbons $\alpha$ to an isocyano nitrogen, which show $^{14}N$ quadrupole couplings due to the low electric field gradient of isocyanides. The presence of 15 skeletal carbons including four methyl groups suggested a sesquiterpene isonitrile, which is typical of this genus of sponge.

The $^1H$ NMR spectrum revealed four methyls as two singlets and two doublets, plus 13 other protons (from integration), which confirmed the presence of 25 protons. C–H correlated 2D NMR spectroscopy[6] established all C–H connectivities and confirmed that two methylene

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[1] A preliminary account was presented at the Gordon Research Conference on Marine Natural Products in Oxnard, CA, 29 February–4 March, 1988.

[2] (a) Hagadone, M. R.; Scheuer, P. J. *J. Am. Chem. Soc.* **1984**, *106*, 2447–2448. (b) Karuso, P.; Scheuer, P. J. *J. Org. Chem.* preceding paper, this issue.

[3] Hagadone, M. R.; Burreson, B. J.; Scheuer, P. J.; Finer, J. S.; Clardy, J. *Helv. Chim. Acta* **1979**, *62*, 2484–2494.

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0022-3263/89/1954-2095$01.50/0

**Table I. NMR Data for 9-Isocyanoneopupukeanane**

| assignment | $^1$H data[a] $\delta$, ppm | mult. | $J$, Hz | $^{13}$C data[b] $\delta$, ppm | mult. |
|---|---|---|---|---|---|
| 1 | 1.54[d] | dddd | 4.2, 3.8, 2.3, 2.2 | 33.8 | d |
| 2 | 2.11[d] | ddd | 14.2, 3.1, 2.2 | 39.9 | t |
| 2′ | 1.07[d] | m | 14.2, 3.8 | | |
| 3 | | | | 39.8 | s |
| 4/5 | 1.20 | m | | 41.7 | 2 t |
| 6 | | | | 39.4 | s |
| 7 | 0.52 | t | 3.2 | 49.1 | d |
| 8 | 1.67 | qd | 15.3, 3.2 | 32.1 | t |
| 8 | 1.67 | qd | 15.3, 3.2 | 32.1 | t |
| 8′ | 1.37 | d (br) | 15.3 | | |
| 9 | | | | 64.5[c] | s |
| 10 | 1.07[d] | m | 14.1, 3.2, 2.2 | 38.8 | t |
| 10′ | 0.88[d] | dd | 14.1, 4.2 | | |
| 11 | 0.66 | s | | 27.1 | q |
| 12 | 1.06 | s | | 26.6 | q |
| 13 | 1.43 | m | | 35.2 | d |
| 14 | 0.67 | d | 6.6 | 16.9 | q |
| 15 | 0.90 | d | 6.6 | 17.4 | q |
| 16 | | | | 158.3[c] | s |

[a] Proton spectra measured in $C_6D_6$ at 300 MHz. [b] Carbon spectra measured in $C_6D_6$ at 125 MHz. [c] For C-9 $J_{NC}$ = 4.7 Hz (triplet); for C-16 $J_{NC}$ = 3.5 Hz (triplet). [d] As the signal for $H_2'$/H10 is second order (though deceptively simple in appearance) the true chemical shifts and coupling constants were obtained from a simulation of the isolated $H_22$, H1, $H_210$ spin system by using the Bruker PANIC routine.

**Table II. COSY, Long-Range COSY, and NOE Connectivities of 9-Isocyanoneopupukeanane**

| $^1$H no. | COSY | long range | NOE |
|---|---|---|---|
| H1 | H2, H2′, H10, H10′ | | |
| H2 | H1, H2′/H10 | H4/5 | |
| H2′/10 | H1, H2, H10′ | H4/5, H7 | no data |
| H4/5 | | H2, H10′,H2′/10, $H_3$11, $H_3$12 | no data |
| H7 | H8, H8′ | H′/10, H10′, $H_3$11, $H_3$12 | H4/5, $H_3$11, $H_3$12 |
| H8 | H7, H8′ | | |
| H8′ | H7, H8 | | |
| H10′ | H1, H2′/10 | H4/5, H7 | H4/5 |
| $H_3$11 | | H7, H2′/10, H4/5 | H4/5, H7, H8′, H2′/10 |
| $H_3$12 | | H7, H2′/10, H4/5 | H2, H4/5, H7, H8 |
| H13 | $H_3$14, $H_3$15 | | H10 |
| $H_3$14 | H13 | $H_3$15 | H8′ |
| $H_3$15 | H13 | $H_3$14 | H1, H10 |

**Table III. Long-Range Carbon–Hydrogen Connectivities in 9-Isocyanoneopupukeanane**

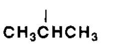
| C no. | proton connectivity | C no. | proton connectivity |
|---|---|---|---|
| 1 | H8′ | 9 | H1, H2′/10, H7, H10′, H2, $H_3$14, $H_3$15 |
| 2 | H4/5, H7, $H_3$12 | 10 | H2, H7, $H_3$11, H8, H8′ |
| 3 | H1, H4/5, H8′, $H_3$12 | 11 | H4/5, H7, H10, H8′ |
| 4/5 | H4/5, $H_3$11, $H_3$12 | 12 | H2, H4/5, H7, H8, H8′ |
| 6 | H1, H7, H8, $H_3$11 | 13 | H8′, $H_3$14, $H_3$15 |
| 7 | H4/5, H8′, $H_3$11, $H_3$12 | 14 | $H_3$15 |
| 8 | H1, H13 | 15 | $H_3$14 |

carbons resonated at 41.7 ppm and that two proton resonances from different carbons overlapped at 1.00 ppm. This allowed the assignment of each carbon from the most downfield (C-16) to the most upfield (C-14) and of all attached protons (Table I).

Homonuclear shift correlated 2D NMR (COSY)[7] spectra provided only a limited amount of structural data; C-2, -1, -10 made up a contiguous set (A) as did C-14, -13, -15, the latter making up an isopropyl group (B). C-7 and C-8 were also adjacent (C), but this was the limit of information to be gained from the standard COSY. However, a COSY experiment optimized for long-range couplings (LRCOSY)[8] revealed a wealth of four and five bond coupling connectivities of less than 1 Hz (see Table II). For instance, H-4/5 is a four-proton multiplet strongly coupled and without large couplings to other protons. This would represent two adjacent methylenes flanked by quaternary carbons (e.g. D). As H-4/5 showed long-range couplings to H-2 and H-10 in the LRCOSY spectrum and also long-range connectivities to C-11 and C-12 in the long-range proton–carbon heteronuclear correlated spectrum (COLOC,[9] Table III), this lead to partial structure E from structures A and D. Now, both H-2′ and H-10′ showed long range (*W*) coupling to H-7 (LRCOSY), while C-3 and C-6 gave strong antiperiplanar correlations to H-8′ and H-8, respectively (COLOC). In addition, C-2 and C-10 were coupled to H-7. Thus the connection between E and C was established to give partial structure F, leaving only C-9, the isocyano group, and the isopropyl group (B) to be accounted for. As C-9 is directly bonded to the isocyano nitrogen (from N coupling in the carbon spectrum) and is quaternary, the isopropyl group, C-1, and C-8 must also be attached to C-9 to result in structure **3**. That C-9 bridges C-1 and C-8 was confirmed by the long-range coupling connectivities from C-9 to H-7 on one side of the bridge and to H-1, H-2′, and H-10′ on the other side (COLOC).

$-CH_2CHCH_2-$

A

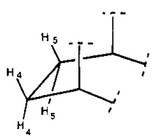


$CH_3\overset{|}{C}HCH_3$

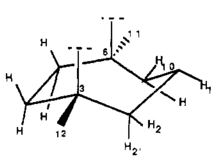
B

$-CHCH_2-$

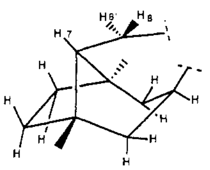
C



D



E



F

Structure **3**, including the relative assignments of $H_3$-11/$H_3$-12, $H_2$-2/$H_2$-10, and H-8/H-8′, was supported by NOE measurements (see Table II); in particular, $H_3$-12 exhibited NOE's to H-2, H-4/5, H-7, and H-8, while $H_3$-11 had NOE's to H-4/5, H7, H-8′, and H-10. The *W* cou-

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[7] (a) Nagayama, K.; Kumar, A.; Wuthrich, K.; Ernst, R. R. *J. Magn. Reson.* **1980**, *40*, 321–334. (b) Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229–2246.

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[9] Kessler, H.; Griesinger, C.; Zarbock, J.; Loosli, H. R. *J. Magn. Reson.* **1984**, *57*, 331–336.

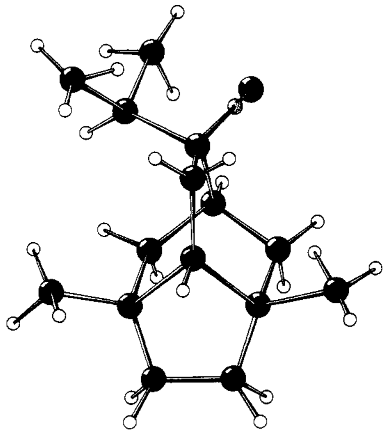


**Figure 1.** Dreiding model of 9-isocyanoneopupukeanane.

plings between H-2 and H-10, H-2 and H-4/5, and H-10 and H-4/5 indicated that H-2 and H-10 are equatorial, while H-2′ and H-10′ must be axial. The connectivity between C-13 and H-8′ (COLOC) rather than H-8 shows that H-8′ and not H-8 has a larger coupling with C-13. This agrees with cis configuration between C-13 and H-8′ and gauche conformation between H-8 and C-13.[10] Thus the relative stereochemistry at C-9 (to that at C-3 and C-6) was deduced from NOE enhancements, which were seen from H-13 to H-10, from $H_3$-14 to H-8′, and also from $H_3$-15 to H-1 and H-10. These data indicated that the isopropyl group is on the same side of the molecule as H-8′, H-10, and $H_3$-11, thus establishing the relative stereochemistry of **3**.

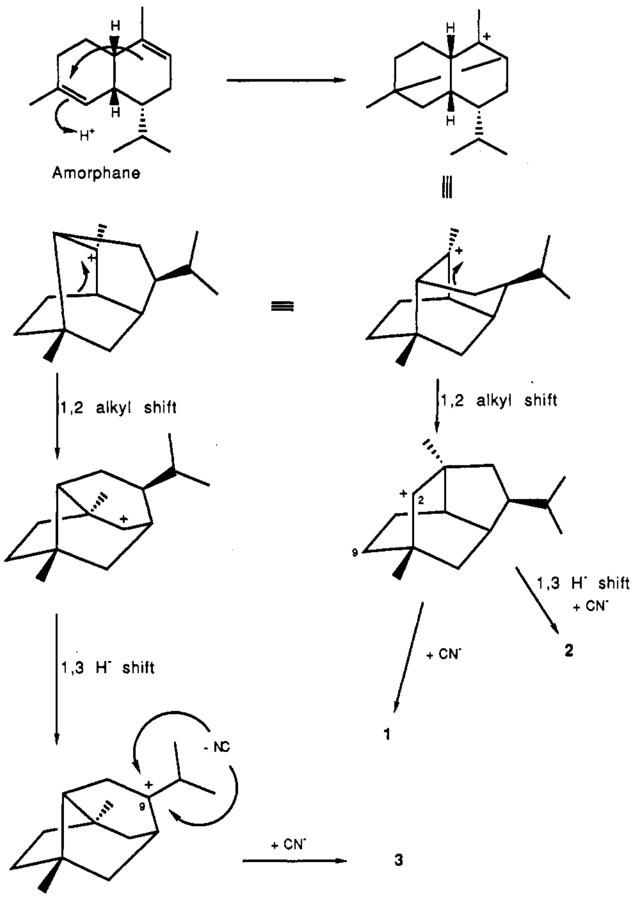
The Dreiding model of **3** (Figure 1) shows that 9-isocyanoneopupukeanane is a beautifully symmetrical compound. Without the isocyano group at C-9, the C-9 carbocation would have a $C_2$ axis and zero optical rotation. Since the terpene presumably is functionalized via a tertiary carbocation, this must be an enantiospecific i.e. enzymatic process. Scheme I illustrates a possible pathway for the genesis of **1**, **2**, and **3** from an amorphane precursor. Amorphane derivatives have been characterized from the north shore *Ciocalypta* sp.[11]

## Experimental Section

Infrared spectra were measured as chloroform solutions on Perkin-Elmer 1420 and Nicolet 5-MX FTIR spectrometers. NMR experiments were performed at 300 MHz on either a Nicolet NT-300 or a General Electric GE-300 instrument and at 500 MHz on a Bruker AM500 instrument. Mass spectra were obtained with a VG 70/SE mass spectrometer. Optical rotations were determined in chloroform on a Rudolph Research Autopol II polarimeter. All solvents were distilled and dried over molecular sieve.

**Isolation of Isocyanoneopupukeanane (3).** Several small specimens of *Ciocalypta* sp. were collected from the Ala Moana reef, on the south shore of O'ahu, HI, at -10 m. The sponge (~200 g) was then blended in ethanol (3 × 200 mL) and filtered. The ethanol filtrates were combined, reduced to half their initial volume, and partitioned against hexane (3 × 150 mL). The hexane partitions were combined and concentrated, yielding a pale yellow residue (760 mg). Chromatography of the residue on BioBeads SX-8 (toluene), normal-phase HPLC (ethanol/hexane, 0.05:99.95), and finally on reversed-phase HPLC (RP-18; acetonitrile/water, 70:30) yielded 30 mg of **3** as a colorless oil: $[\alpha]_D$ +33° (*c* 1.0, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 2910, 2870, 2800, 2115, 1695, 1640, 1430, 1420, 1335 cm$^{-1}$; 300-MHz $^1H$ NMR, see Table I; $^{13}C$ NMR ($C_6D_6$) $\delta$ 16.9 (q, $J$ = 126.1 Hz), 17.4 (q, $J$ = 125.9 Hz), 26.6 (q, $J$ = 128.0 Hz), 27.1 (q, $J$ = 125.2 Hz), 32.1 (t, $J$ = 128.0 Hz), 33.8 (d, $J$ = 135.9 Hz), 35.2 (d, $J$ = 126.6 Hz), 38.8 (t, $J$ = 122.9 Hz), 39.4 (s), 39.8 (s), 39.9 (t, $J$ = 125.9 Hz), 41.7 (2 t, $J$ = 128.1 Hz), 49.1 (d, $J$ = 129.3 Hz), 64.5 (t, $J_{NC}$ = 4.7 Hz), 158.3 (t, $J_{NC}$ = 3.5 Hz); EIMS *m/z* (relative intensity) 231 ($M^+$, 8.5), 216 (17), 204 (39), 189 (100), 161 (39), 147 (39), 133 (29), 121 (30), 119 (40), 108 (47), 107 (59), 105 (49), 95 (77), 93 (82), 91 (50), 81 (40), 55 (45). Anal. Calcd for $C_{16}H_{25}N$: 231.1987. Found: 231.1988.

**Scheme I**



**Acknowledgment.** We thank Greg Standal for assistance with collections, Jennifer Hill for assistance with the chromatography of **3**, and A. I. Scott for the use of 500-MHz NMR instrumentation, and the National Science Foundation and the University of Hawaii Sea Grant College Program under Institutional Grant NA81AA-D-0070 from NOAA, Office of Sea Grant, U.S. Department of Commerce, for financial support.

**Registry No.** **3**, 119323-93-2.

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