(relative intensity) 143 (3.3), 129 (55.5), 110 (9.7), 98 (31.1), 87 (100), 69 (51.2), 55 (38.1), 45 (44.1), 41 (41.4), 29 (43.7), high-resolution mass spectrum, calcd for $C_{10}H_{22}O - C_2H_5$ 129.1280, found 129.1260.

Reaction of 6, R = Methyl. The same procedure with 7 was followed, except 1.0 equiv of MeLi (0.32 mL, 0.50 mmol, 1.58 M) was added to CuCN. VPC analysis showed formation of 4 in 6% yield.

Reaction of 6, R = *n*-Butyl. The procedure given for the preparation of 8 was followed except 1.0 equiv of *n*-BuLi (0.20 mL, 0.50 mmol, 2.50 M) was added to CuCN. GC analysis indicated that 5 had been formed to the extent of ca. 3%.

Preparation and Reaction of 9 with Epoxide 3. Copper cyanide (44.8 mg, 0.50 mmol) was placed in a 25-mL two-neck round-bottom flask which contained a magnetic stir bar. The salt was dried azeotropically with toluene (1 × 1.0 mL) under vacuum at room temperature and then purged with dry argon. Dry THF (1.0 mL) was introduced producing a slurry which was cooled to −78 °C. Methyllithium (0.32 mL, 0.50 mmol, 1.58 M) was added dropwise which resulted in a light tan solution after ca. 10 min. Subsequent addition of *n*-butyllithium (0.20 mL, 0.50 mmol, 2.50 M) produced no visible change. After warming the solution to −40 °C, the oxirane 3 (59 μL, 0.50 mmol) was added neat via syringe. Stirring was maintained at this temperature for 2 h after which time the reaction was quenched with 5 mL of a 90% saturated NH_4Cl/10% concentrated NH_4OH solution. After stirring for an additional 30 min at room temperature, the mixture was transferred to a separatory funnel and extracted with Et_2O (2 × 5 mL). VPC analysis indicated both 4 and 5 were present in yields of 8.1% and 61.8%, respectively, with 13% starting material remaining.

Preparation, Mixing, and Reaction of 7 and 8 with Epoxide 3. Cuprous cyanide (22.4 mg, 0.25 mmol) was placed in a 25-mL two-neck round-bottom flask equipped with a magnetic stir bar. After the salt was dried azeotropically with toluene (1 × 1.0 mL) under vacuum at ambient temperature, the vessel was purged with and maintained under dry argon. THF (0.50 mL) was added and the slurry was cooled to −78 °C where *n*-BuLi (0.20 mL, 0.50 mmol, 2.5 M) was introduced dropwise generating a light tan solution within 10 min.

In a separate flask, the above procedure was duplicated substituting methyllithium (0.32 mL, 0.50 mmol, 1.58 M) for *n*-BuLi, which likewise produced a light tan solution.

Reagent 7 ($Me_2Cu(CN)Li_2$), precooled to −78 °C, was transferred via cannula into cuprate 8, which was also maintained at −78 °C. The resulting solution was stirred for 5 min before warming to −40 °C. Epoxide 3 (54 μL, 0.50 mmol) was then added dropwise with stirring upon completion of which the reaction was continued for 2 h before quenching with 5 mL of a 90% saturated NH_4Cl/10% concentrated NH_4OH solution. Following an additional 30-min period of stirring at room temperature, extractive workup (Et_2O, 2 × 5 mL) was followed by VPC analysis which showed that both products 4 and 5 were present in 8.6% and 60.3% yields, respectively.

Representative Procedure for NMR Sample Preparation. $Me_2Cu(CN)Li_2$. Following the generation of 7 as outlined above, the cold (−78 °C) solution was transferred under Ar via cannula to a dry, cold NMR tube. The tube was then fitted with a teflon plug from which a capillary insert containing CH_2CH_2/acetone-d_6 was suspended. The spectrum was then immediately recorded. The same technique was applied to both 9 (prepared from sequential addition of MeLi and *n*-BuLi) and 9 (prepared by mixing 1 equiv each of 7 and 8).

Preparation of 7 Containing 1 Equiv of *n*-BuLi. To cuprate 7 formed as described above and cooled to −78 °C was added 1.0 equiv of *n*-BuLi. The solution was then transferred via cannula to an NMR tube in the usual fashion and the spectrum immediately taken.

NMR Analysis of Reaction of 7 with 1-Methylcyclopentene Oxide. To reagent 7, cooled to 0 °C and prepared as described above, was added 1.0 equiv of 1-methylcyclopentene oxide[38] heat via syringe. Aliquots were transferred to an NMR tube during the course of the reaction (after 0.75, 3, and 6 h) in the usual fashion.

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Registry No. 3, 1192-17-2; 4, 597-49-9; 5, 2051-32-3; 6 (R = Me), 41753-78-0; 6 (R = *n*-Bu), 41742-63-6; 7, 80473-70-7; 8, 80473-69-4; 9, 86250-96-6; *t*-BuLi, 594-19-4; Me_2CuLi, 15681-48-8; *t*-Bu_2CuLi, 24406-16-4; CuSCN, 1111-67-7; $Me_2Cu(SCN)Li_2$, 91606-28-9; PhLi, 591-51-5; $Me(Ph)Cu(CN)Li_2$, 91328-62-0; *n*-BuMgCl, 693-04-9; MeLi, 917-54-4; *n*-BuLi, 109-72-8; *n*-$Bu_2Cu(CN)(MgCl)_2$, 91606-30-3; cyclopentyl iodide, 1556-18-9; 2-bromopentane, 107-81-3; cuprous cyanide, 544-92-3; 1-methylcyclopentene oxide, 16240-42-9.

Supplementary Material Available: Figures 1, 3, 6–9, and IR data for ref 29 (7 pages). Ordering information is given on any current masthead page.

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New Nitrogenous Sesquiterpenes from the Marine Sponge *Axinella cannabina*

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In addition to acanthellin-1 (1), an isonitrile previously found in *Acanthella acuta*, five new nitrogenous sesquiterpenes (2–6) have been isolated from the Mediterranean sponge *Axinella cannabina* and their structures elucidated on the basis of chemical and spectral data. These metabolites form two new isocyanide–isothiocyanate–formamide series thus confirming the biogenetical relationship between these three classes of compounds.

The marine sponge *Axinella cannabina* is a fertile source of sesquiterpenes with diverse and often unusual carbocyclic skeleton, carrying isocyanide, isothiocyanate, or formylamine functionalities.[1-5] In view of the potential

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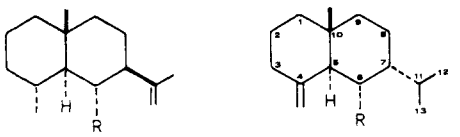
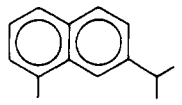
biological interest of these classes of compounds,[6] we have further investigated the chloroform soluble extract from this organism and, after extensive chromatography, we have found five new nitrogenous sesquiterpenes (2–6) in addition to acanthellin-1 (1), an iscyanide previously isolated from *Acanthella acuta*, a sponge belonging to the same family.[7]



1 R = $\overset{+}{N}\equiv\dot{C}$ 4 R = $\overset{+}{N}\equiv\dot{C}$

2 R = N=C=S 5 R = N=C=S

3 R = N−C−H (H, O) 6 R = N−C−H (H, O)



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Isonitrile **4**, $[\alpha]_D$ +39.6, had the molecular formula $C_{16}H_{25}N$ (HRMS). The IR spectrum contained an isonitrile band at 2135 cm^{-1} and two further absorptions at 1640 and 895 cm^{-1}, indicative of an exo methylene group, which was confirmed by the presence of two typical 1 H narrow multiplets in the ^1H NMR spectrum at δ 5.05 and 4.72. The spectrum also included a methyl singlet at δ 0.79 and two secondary methyl signals δ 1.16 and 1.04 (J = 6.5 Hz), belonging to an isopropyl group since they collapsed into two singlets by irradiation at δ 2.18 (1 H, octet, J = 6.5 Hz, H-11).

This spectral evidence suggested that the compound under investigation was a bicyclic sesquiterpene carrying an isonitrile, an isopropyl, and a tertiary Me group. Additional proof for the structure of **4** was obtained by its dehydrogenation in the presence of 10% Pd/C, which afforded eudalene (**7**) in good yields, thus establishing the nature of the bicyclic skeleton and the position of the isopropyl and exo methylene groups.

Further analysis of ^1H NMR spectrum of **4** and extensive double resonance experiments showed that the isonitrile group was located at C-6 and that the tertiary methyl must be linked to C-10. In fact, the methyne H-6 signal at δ 3.91, which appeared as a double doublet (J = 11.5 and 5.0 Hz), each line being split into a 1:1:1 triplet (J = 2.5 Hz) by coupling with the nitrogen atom, was proven to be coupled with the doublet at δ 2.34 (1 H, J = 11.5 Hz, H-5 broadened by long range coupling with the vinyl protons) and the multiplet at δ 2.03 (H-7) in turn coupled with the isopropyl proton at δ 2.18. The values of the coupling constants required a diaxial relationship between H-6 and H-5 and an equatorial nature of H-7, thus establishing the relative stereochemistry of **4** apart from the chirality at C-10. This was deduced by the presence of a nuclear Overhauser effect between Me-10 and H-6.

Isothiocyanates 2 and 5. Compound **2**, $[\alpha]_D$ −24.4, had the molecular formula $C_{16}H_{25}NS$ (HRMS). Spectral data [ν_{max} 2100, 1640, 1375, 1365, 895 cm^{-1}; ^1H NMR δ 4.95 and 4.92 (1 H each, narrow ms of vinyl protons), 3.46 (1 H, t, J = 10.5 Hz, H-6), 1.77 (3 H, bs, Me-11), 1.14 (3 H, d, J = 6.5 Hz, Me-4), and 0.86 (3 H, s, Me-10)] indicated a close relationship between **2** and acanthellin-1 (**1**) as proven by the following: compound **1** by treatment with sulfur at 120 °C afforded the corresponding isothiocyanate shown to be identical with **2** by comparison of their chromatographic and spectral properties.

The isothiocyanate **5**, $[\alpha]_D$ +41.0, had the molecular formula $C_{16}H_{25}NS$ (HRMS). The IR spectrum contained a strong isothiocyate band at 2100 cm^{-1}. The ^1H NMR spectrum [δ 5.04 and 4.64 (1 H each, narrow ms, vinyl protons), 2.33 (1 H, bd, J = 11.5 Hz, H-5), 2.05 (1 H, octet, J = 6.5 Hz, H-11), 1.86 (1 H, m, H-7), 1.14 and 1.01 (3 H each, ds, J = 6.5, Hz, H_3-12 and H_3-13), and 0.80 (3 H, s, Me-10)] was almost identical with that of isonitrile **4**, with the exception that the H-6 signal was now a sharp double doublet (δ 4.09, J = 11.5 and 5.0 Hz) lacking the coupling with the nitrogen atom which is peculiar of isocyanides. The correlation between the two compounds was confirmed by conversion of **4** into **5** by treatment with sulfur.

Formamides 3 and 6. Isonitriles in marine sponges are generally accompanied by the corresponding isothiocyanates and formamides and this was considered to be evidence of the strict biogenetic relationship between these three classes of compounds. Formamides **3** and **6** are actually constituents of *A. cannabina* and they were isolated as pure products from the most polar fraction of the chloroform extract.

Amide **3** [oily; ν_{max} 3440, 1680 cm^{-1} (amide); $[\alpha]_D$ −24.0] had the molecular formula $C_{16}H_{27}NO$ (HRMS). In the ^1H NMR spectrum, signals due to two rotational isomers of the formamide group are present in the ratio 5 (cis):2 (trans). Cis isomer: ^1H NMR δ 7.99 (d, J = 2.2 Hz, HC=O), 4.88 (b, >NH), 4.70 and 4.64 (narrow ms, >C=CH_2), 4.09 (q, J = 10.2 Hz, H-6), 1.70 (s, Me-11), 0.95 (d, J = 6.5 Hz, Me-4), 0.93 (s, Me-10). Trans isomer: ^1H NMR δ 7.77 (d, J = 12.4 Hz, HC=O), 5.13 (b, >NH), 4.86 and 4.74 (narrow ms, >C=CH_2), 3.26 (q, J = 10.2 Hz, H-6), 1.68 (s, Me-11), 0.91 (s, Me-10), and 0.85 (d, J = 6.5 Hz, Me-4).

The above assignments were confirmed by extensive spin decoupling experiments. The structure of **3** was definitively proven by comparison of its spectral and chromatographic properties with those of a sample synthesized from acanthellin-1 by treatment with AcOH in Et_2O.

Amide **6** [oily; ν_{max} 3440, 1675 cm^{-1} (amide); $[\alpha]_D$ +48.5] had the molecular formula $C_{16}H_{27}NO$ (HRMS).

In contrast to **3**, amide **6** exists almost exclusively as the cis isomer at the formamide group: ^1H NMR δ 8.08 (1 H, d, J = 1.5 Hz, HC=O), 5.30 (1 H, b, >NH), 4.89 and 4.43 (1 H each, narrow ms, >C=CH_2), 4.22 (1 H, ddd, J = 11.2, 9.0, and 4.5 Hz, H-6), 1.00 and 0.95 (3 H each, ds, J = 6.5 Hz, H_3-12 and H_3-13), and 0.84 (3 H, s, Me-10). The identity of **6** was confirmed by its synthesis by hydration of **4**.

The isolation from *A. cannabina* of two formamide–isothiocyanate–isocyanide series supports the argument that these three functionalities are biogenetically related and shows the capability of this marine organism to elaborate a large number of sesquiterpene skeletons.

Experimental Section

IR spectra (CCl_4) were recorded on a Perkin Elmer 157 spec-

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trophotometer. NMR spectra were determined on a Bruker WM-250 spectrometer in $CDCl_3$ solutions with Me_4Si as internal reference (δ 0). Nuclear Overhauser effect was determined with the aid of an Aspect 2000 microprogram which allowed direct accumulation of NOE difference FIDs on a sample degassed by bubbling Ar through the solution for 40 min.

Mass spectra were taken on AEI 902 instrument. Optical rotations were measured with a Perkin Elmer 141 polarimeter with a 10-cm microcell.

Extraction. Sponge (*Axinella cannabina*), collected in the Bay of Taranto, near Porto Cesareo in Autumn 1983 (350 g, dry after extraction), was homogenized and extracted four times with CH_3OH at room temperature for 3 days. The combined extracts (5 L) were concentrated under red press and the remaining aqueous residue was extracted with $CHCl_3$. The organic phase was taken to dryness leaving an oily residue (15 g) which was chromatographed on a SiO_2 (900 g) column under pressure, using as eluent solvent mixtures in increasing polarities from 40–70 °C light petroleum to Et_2O through benzene. Elution with 40–70 °C light petroleum–benzene (8:2), 40–70 °C light petroleum–benzene (2:8), and Et_2O afforded fractions A (1.6 g), B (5 g), and C (300 mg) respectively, which were used for the isolation of 1–6 as described below.

Isolation of 2 and 5. Fraction A (1.6 g), obtained as above, was rechromatographed on a silica gel column (100 g), eluent 40–70 °C light petroleum, to give 300 mg of an oily residue containing a mixture of isothiocyanates, which were separated by HPLC (LiChrosorb Si 60, *n*-hexane), thus obtaining 18 mg of **2** and 20 mg of **5**: $[\alpha]_D$ + 41.0 (*c* 0.35, $CHCl_3$); HRMS, *m/z* 263.1701, $C_{16}H_{25}NS$ requires 263.1709; ν_{max} 2100, 1640, 895 cm^{-1}; δ 5.04 and 4.64 (1 H each, narrow ms), 4.09 (1 H, dd, *J* = 11.5 and 5.0 Hz), 2.33 (1 H, bd, *J* = 11.5 Hz), 2.05 (1 H, octet, *J* = 6.5 Hz), 1.86 (1 H, m), 1.14 and 1.01 (3 H each, ds, *J* = 6.5 Hz), and 0.80 (3 H, s). **2**: $[\alpha]_D$ −24.4 (*c* 0.28, $CHCl_3$); HRMS, *m/z* 263.1706, $C_{16}H_{25}NS$ requires 263.1709; ν_{max} 2100, 1640, 895 cm^{-1}; δ 4.95 and 4.92 (1 H each, narrow ms), 3.46 (1 H, t, *J* = 10.5 Hz), 1.77 (3 H, bs), 1.14 (3 H, d, *J* = 6.5 Hz), and 0.86 (3 H, s).

Isolation of 1 and 4. Rechromatography of fraction B (5 g), obtained as above, with 40–70 °C light petroleum–benzene 8:2 on a silica gel column (400 g), gave an oily product (503 mg), which was further purified by HPLC (μ-bondapack C_{18}, eluent 20% H_2O in CH_3OH), thus affording 20 mg of **4** [$[\alpha]_D$ +39.6 (*c* 0.30, $CHCl_3$); ν_{max} 2135, 1640 and 895 cm^{-1}; HRMS, *m/z* 231.1985, $C_{16}H_{25}N$ requires 231.1988; δ 5.05 and 4.72 (1 H, narrow ms), 3.91 (1 H, ddt, *J* = 11.5, 5.0, and 2.5 Hz), 2.34 (1 H, d, *J* = 11.5 Hz), 2.18 (1 H, octet, *J* = 6.5 Hz), 2.03 (1 H, m), 1.16 (3 H, d, *J* = 6.5 Hz), 1.04 (3 H, d, *J* = 6.5 Hz), 0.79 (3 H, s)] and 22 mg of **1** [$[\alpha]_D$ −42.3 (*c* 0.53, $CHCl_3$)], which was identical in all aspects with authentic sample.[7]

Isolation of 3 and 6. Fraction C (300 mg), obtained as above, was chromatographed on a silica gel column (80 g, eluent Et_2O), thus obtaining a mixture of **3** and **6**, which was resolved into the individual components by HPLC (LiChrosorb Si 60, EtOAc). **3** (4 mg): $[\alpha]_D$ −24.0 (*c* 0.30, $CHCl_3$); ν_{max} 3440 and 1680 cm^{-1}; HRMS, *m/z* 249.2089, $C_{16}H_{27}NO$ requires 249.2094. Cis isomer: δ 7.99 (d, *J* = 2.2 Hz), 4.88 (1 H, b), 4.70 and 4.64 (1 H each, narrow ms), 4.09 (1 H, q, *J* = 10.2 Hz), 1.70 (3 H, s), 0.95 (3 H, d, *J* = 6.5 Hz), 0.93 (3 H, s). Trans isomer: δ 7.77 (d, *J* = 12.4 Hz), 5.13 (1 H, b), 4.86 and 4.74 (1 H each, narrow ms), 3.26 (1 H, q, *J* = 10.2 Hz), 1.68 (3 H, s), 0.91 (3 H, s), and 0.85 (3 H, d, *J* = 6.5 Hz). **6** (3.5 mg): $[\alpha]_D$ +48.5 (*c* 0.30, $CHCl_3$); ν_{max} 3440 and 1675 cm^{-1}; HRMS, *m/z* 249.2086, $C_{16}H_{27}NO$ requires 249.2094. Cis isomer: 8.08 (1 H, d, *J* = 1.5 Hz), 5.30 (1 H, b), 4.89 and 4.43 (1 H each, narrow ms), 4.22 (1 H, ddd, *J* = 11.2, 9.0, and 4.5 Hz), 1.00 and 0.95 (3 H each, ds, *J* = 6.5 Hz), and 0.84 (3 H, s).

Treatment of 1 with Sulfur To Obtain 2. Compound **1** (5 mg) and excess S were heated at 120 °C for 16 h; after addition of 40–70 °C light petroleum (5 mL) and filtration, the solution was taken to dryness and the residue was purified by TLC (silica gel, *n*-hexane). The band R_f 0.6, scraped and eluted with Et_2O, afforded 3 mg of an oily product, which was identical with natural **2** on the basis of their chromatographic and spectral properties.

Treatment of 4 with Sulfur To Obtain 5. This was analogous to the preparation of **2** from **1** above. From 5 mg of **4**, 4 mg of crude **5** were obtained. After purification by TLC (SiO_2, *n*-hexane), its spectral and chromatographic properties matched those of natural **5**.

Hydration of 1 To Obtain 3. A solution of **1** (12 mg) in anhydrous Et_2O (6 mL) and AcOH (5 mL) was kept at room temperature for 2 h. After washing with 10% aqueous Na_2CO_3 and then with H_2O, the organic phase was dried and taken to dryness, thus giving a residue which was chromatographed on TLC (SiO_2, Et_2O); the band R_f 0.5, eluted with Et_2O, gave a compound (7.5 mg), which was identical with natural **3** by comparison of spectral and chromatographic data.

Hydration of 4 To Give 6. Compound **4** (8 mg) was treated with AcOH (5 mL) in anhydrous Et_2O (6 mL) by the procedure used for **1**, thus **6** (4.5 mg) was obtained identical with **6** isolated from *A. cannabina*.

Dehydrogenation of 4 to Eudalene 7. A mixture of **4** (5 mg) and 10% Pd/C (10 mg) was heated at 280 °C under N_2 for 1 h. Extraction of the mixture with $CHCl_3$ and TLC (silica gel, *n*-hexane, UV) gave 2 mg of eudalene identified by comparison of its spectral properties with those of an authentic sample.

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Registry No. **1**, 54462-52-1; **2**, 91466-60-3; **3**, 91466-61-4; **4**, 91466-62-5; **5**, 91466-63-6; **6**, 91466-64-7; **7**, 490-65-3.

Regio- and Stereoselective Synthesis of Methyl α-L-Daunosaminide Hydrochloride

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Methyl 4-*O*-(trichloroacetamidoyl)-2,3,6-trideoxy-α-L-*threo*-hex-2-enopyranoside (**6**), prepared from methyl 2,3,6-trideoxy-α-L-*threo*-hex-2-enopyranoside (**5**), was cyclized to the iodooxazoline **7** which, by hydrolysis under acidic conditions, afforded the corresponding hydrochloride **8**. The deiodination reaction was performed with Bu_3SnH and methyl 3-amino-2,3,6-trideoxy-α-L-lyxopyranoside hydrochloride (methyl α-L-daunosaminide hydrochloride) **1** was obtained in 80% yield. The intermediate **5** was synthesized starting from methyl 4-*O*-mesyl-2,3,6-trideoxy-α-L-*erythro*-hex-2-enopyranoside **3** in 80% yield by means of carbonate anion on polymeric support.

3-Amino-3-deoxyhexoses have been widely encountered as sugar moieties of biologically active substances. L-Daunosamine (3-amino-2,3,6-trideoxy-L-*lyxo*-hexose), in particular, is important as the carbohydrate constituent

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