(6*R*,7*S*)-7-Amino-7,8-dihydro-α-bisabolene, an Antimicrobial Metabolite from the Marine Sponge *Halichondria* sp.

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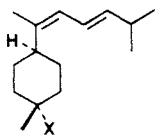
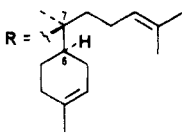
The antimicrobial constituents of the marine sponge *Halichondria* sp. from Ponape have been identified as (6*R*,7*S*)-7-amino-7,8-dihydro-α-bisabolene (**3**) and its hydrochloride salt **4**. The structure of amine **3** was determined by an X-ray crystallographic study of the corresponding symmetrical urea **5** and the absolute configuration was elucidated by chemical conversion to (6*R*,7*E*)-α-bisabolene. The urea **5**, the corresponding isothiocyanate **6**, and an unrelated diene **7** were also isolated from the sponge.

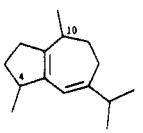
A number of sesquiterpene isonitriles, isothiocyanates, and formamides and diterpene diisonitriles have been isolated from sponges of the order Halicondria.[1] Recently, Nakamura et al. described theonellin isothiocyanate (**1**) and theonellin formamide (**2**), but not the corresponding isonitrile, from the Okinawan sponge *Theonella* cf. *swinhoei*.[2] The theonellin derivatives are sesquiterpenes of the bisabolene class with nitrogen substitution at C-3. We report the isolation of the antimicrobial metabolite (6*R*,7*S*)-7-amino-7,8-dihydro-α-bisabolene (**3**), its hydrochloride salt **4**, the corresponding urea **5** and isothiocyanate **6**, and an unrelated sesquiterpene hydrocarbon **7** from the sponge *Halichondria* sp.



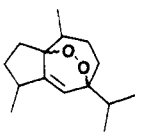
1 X = NCS
2 X = NHCHO



3 RNH_2
4 $RNH_3^+ Cl^-$
5 RNHCONHR
6 RNCS



7



8 a,b

Halichondria sp. was collected from six locations on the fringing reef at Ponape, Marshall Islands. The samples looked similar but since there were minor variations of color and growth form, they were examined separately. The samples all contained essentially the same mixture of metabolites and the crude extracts all exhibited the same antimicrobial spectrum. Extraction and solvent partition resulted in a hexane extract that contained the majority of the material and antimicrobial activity, with a minor amount of antimicrobial material in the dichloromethane extract. Chromatographic separation resulted in the isolation of the diene **7** (0.48% dry weight), an inseparable mixture of two unstable isonitriles, the urea **5** (0.43% dry weight), the isothiocyanate **6** (0.12% dry weight), (6*R*,7*S*)-7-amino-7,8-dihydro-α-bisabolene (**3**, 0.69% dry weight), and the corresponding hydrochloride salt **4** (3.05% dry weight). The isonitriles decomposed during attempted purification. The amine **3** and the corresponding hydrochloride salt **4** were readily interconverted.

The amine **3**, $[\alpha]_D$ +60°, was isolated as an oil. The molecular formula, $C_{15}H_{27}N$, was obtained from high resolution mass spectrometry data. The ^1H NMR spectrum contained methyl signals at δ 1.03 (s, 3 H), 1.61 (s, 3 H), 1.64 (s, 1 H), and 1.68 (s, 3 H) and two olefinic proton signals at 5.10 (br t, 1 H, J = 7 Hz) and 5.39 (br s, 1 H). Since the ^{13}C NMR spectrum contained only four olefinic carbon signals at δ 133.4 (s), 130.6 (s), 125.8 (d), and 121.8 (d), the amine must contain a $(CH_3)_2C{=}CHCH_2{-}$ group and a trisubstituted olefin with one methyl substituent. Furthermore, the ^{13}C NMR signal at δ 52.8 (s) suggested that the tertiary methyl group was located on a carbon-bearing nitrogen. Comparison of these data with the spectral data of the bisabolenes strongly suggested a 7-amino-7,8-dihydro-α-bisabolene structure. This proposal was confirmed by Hofmann degradation of the amine **3** to produce a mixture of bisabolenes from which (6*R*,7*E*)-α-bisabolene was obtained after chromatography on silver nitrate impregnated silica gel.

The stereochemistry of the amine was established by an X-ray crystallographic study of the corresponding urea **5**. Treatment of the amine **3** with carbonyl diimidazole in THF at 25 °C for 16 h gave the urea, mp 139–141 °C, identical in all respects, including optical rotation, with the natural material. A computer-generated drawing of the final X-ray model of the urea **5** is given in Figure 1. Hydrogens are omitted for clarity and the absolute configuration was determined as indicated previously. The crystal structure is slightly more complicated than that shown in Figure 1. Space group $P4_2$ has a crystallographic twofold axis, and this symmetry element is used by the urea. The twofold axis lies along the C=O bond, and one-half of the molecule is derived from the other half by a twofold rotation. Thus, in the drawing, only half of the atoms are crystallographically unique. There is another, crystallographically independent molecule in the unit cell. Since the conformation and configuration of both independent molecules are the same, only one is shown. Due to the paucity of observed data, the accuracy of this determination is not high. The agreement between the observed geometry and generally accepted values is acceptable.

The isothiocyanate **6** is an oil of molecular formula $C_{16}H_{25}NS$. A band at 2130 cm^{-1} in the IR spectrum indicates the presence of an isothiocyanate group. The ^1H NMR spectrum contains two olefinic proton signals at δ 5.38 (br s, 1 H), 5.09 (br t, 1 H, J = 7 Hz), three olefinic

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(2) Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1984**, *25*, 5401.

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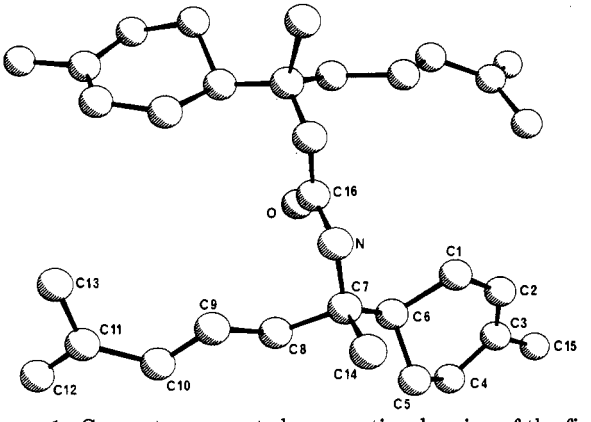

Figure 1. Computer-generated perspective drawing of the final X-ray model of urea **5**. Hydrogens are omitted for clarity, and the absolute configuration was determined by degradation to a compound of known absolute configuration.

methyl signals at 1.69 (s, 3 H), 1.65, (s, 3 H), and 1.63 (s, 3 H), and a signal at 1.34 (s, 3 H) that is assigned to a methyl group bearing an isothiocyanate group. Comparison of the ^{13}C NMR spectra of isothiocyanate **6** and amine **3** indicated that both had the bisabolene carbon skeleton and that the isothiocyanate **6** is therefore 7-isothiocyanato-7,8-dihydro-α-bisabolene.

We were surprised to find that the hydrocarbon **7** was totally unrelated to the bisabolenes. The hydrocarbon **7**, $[\alpha]_D$ −30.2°, had the molecular formula $C_{15}H_{24}$. The ^{13}C NMR signals at δ 151.1 (s), 142.1 (s), 135.9 (s), and 117.1 (d), together with the UV absorption at 268 nm (ε 6700), indicated the presence of a highly substituted homoannular diene in a bicyclic molecule. The ^1H NMR spectrum contained methyl doublets at δ 1.01 (d, 3 H, J = 7 Hz), 1.02 (d, 6 H, J = 7 Hz), and 1.03 (d, 3 H, J = 7 Hz) and a single olefinic proton signal at 5.52 (s, 1 H). Irradiation of an allylic proton signal at δ 2.31 (m, 1 H) caused the doublet at δ 1.02 to collapse to a singlet, indicating the presence of an allylic isopropyl group. Treatment of the hydrocarbon **7** with palladium on carbon in refluxing xylene solution gave guaiazulene. The hydrocarbon **7** is therefore a guaia-1(5),6-diene. The homoannular diene reacted cleanly with singlet oxygen in 5% methanol in dichloromethane solution to obtain two cyclic peroxides **8a** and **8b**. Attempts to elucidate the stereochemistry of the methyl groups at C-4 and C-10 by interpretation of lanthanide-induced shifts in the ^1H NMR spectra of the peroxides **8a** and **8b** gave ambiguous results.

In preliminary assays, both the amine **3** and its hydrochloride salt **4** inhibited the growth of *Staphylococcus aureus*, *Bacillus subtilis*, and *Vibrio anguillarum* at 100 μg/disk but inhibited *Candida albicans* at <25 μg/disk. The amine **3** inhibited *C. albicans* (MIC 5 μg/mL) and *Trichophyton mentagrophytes* (MIC 125 μg/mL) in vitro but was ineffective or toxic in an in vivo screen. The amine **3** was also mildly cytotoxic (CCRF-CEM IC_{50} = 3.1 μg/mL). The diene **7** inhibited the growth of *S. aureus* at 25 μg/mL and *B. subtilis* at 100 μg/mL.

Experimental Section

Typical Isolation Procedure. The frozen sponge (60 g dry weight) was cut into pieces (~8 cm^3) and soaked in methanol (sufficient to cover) for 1 day. The solvent was decanted and soaking repeated. The combined methanol extracts were concentrated to obtain an aqueous suspension that was extracted sequentially with hexane (3 × 200 mL), dichloromethane (3 × 250 mL), and ethyl acetate (3 × 250 mL). The antimicrobial activity was concentrated in the hexane extract, which was subjected to flash chromatography on silica gel. A fraction eluted with hexane contained the diene **7** (287 mg, 0.48% dry weight). The isothiocyanate **6** (76 mg, 0.12% dry weight) was eluted with 1% ether in hexane while the fraction eluted with 5% ether in hexane (60 mg) contained an inseparable mixture of two unstable isonitriles. The urea **5** (260 mg, 0.43% dry weight) was eluted with 10% ether in hexane. Surprisingly, the amine hydrochloride **4** (1.814 g, 3.05% dry weight) was eluted with 0–5% ethyl acetate in ether while the amine **3** (411 mg, 0.69% dry weight) required ethyl acetate for elution. An analytical sample of each compound was obtained by LC on Partisil using an appropriate solvent system.

(6*R*,7*S*)-7-Amino-7,8-dihydro-α-bisabolene (3): $[\alpha]^{20}_D$ +59.9° (*c* 3.0, $CHCl_3$); IR (CCl_4) 2940, 1680, 1440, 1336 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.03 (s, 3 H), 1.61 (s, 3 H), 1.64 (s, 3 H), 1.68 (s, 3 H), 2.00 (m, 4 H), 5.10 (br t, 1 H, J = 7 Hz), 5.39 (br s, 1 H); ^{13}C NMR (C_6D_6) δ 133.4 (s), 130.6 (s), 125.8 (d), 121.8 (d), 52.8 (s), 43.6 (d), 41.2 (t), 31.7 (t), 26.4 (t), 25.8 (q), 25.4 (q), 24.2 (t), 23.5 (q), 22.8 (t), 17.6 (q); FDMS obsd, m/z 221.2128, $C_{15}H_{27}N$ requires m/z 221.2143.

(6*R*,7*S*)-7-Amino-7,8-dihydro-α-bisabolene hydrochloride (4): $[\alpha]^{20}_D$ +64.1° (*c* 4.2, $CHCl_3$); IR (CCL_4) 2910, 1615, 1555, 1540, 1515 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.37 (s, 3 H), 1.61 (s, 6 H), 1.66 (s, 3 H), 5.06 (br t, 1 H, J = 7 Hz), 5.33 (br s, 1 H), 8.34 (bs, 2 H, D_2O exchangeable); ^{13}C NMR (C_6H_6) 134.0 (s), 132.5 (s), 124.7 (d), 121.0 (d), 60.3 (s), 41.3 (d), 37.0 (t), 31.4 (t), 27.2 (t), 26.3 (q), 24.5 (t), 23.9 (q), 23.0 (t), 22.3 (q), 18.4 (q); FDMS, m/z 221.

***N*,*N*'-Bis[(6*R*,7*S*)-7,8-dihydro-α-bisabolen-7-yl]urea (5)**: mp 139–141 °C; $[a]^{20}_D$ +44° (*c* 1.1, $CHCl_3$); IR ($CHCl_3$) 3540, 2910, 1680, 1510, 1210 cm^{-1}; ^1H NMR ($CDCl_3$) 1.07 (s, 6 H), 1.59 (s, 6 H), 1.62 (s, 6 H), 1.67 (s, 6 H), 3.84 (s, 2 H, D_2O exchangeable), 5.12 (br t, 2 H, J = 7 Hz), 5.35 (br s, 2 H); ^{13}C NMR ($CDCl_3$) 156.4 (s), 133.9 (s), 131.3 (s), 124.9 (d), 121.0 (d), 57.4 (s), 41.2 (d), 36.8 (t), 31.4 (t), 26.6 (t), 25.8 (q), 24.6 (t), 23.3 (q), 22.7 (t), 21.4 (q), 17.6 (q); HRMS, obsd m/z 468.4080, $C_{31}H_{52}N_2O$ requires m/z 468.4080.

7-Isothiocyanato-7,8-dihydro-α-bisabolene (6): oil; $[\alpha]^{20}_D$ +60.5° (*c* 6.8, $CHCl_3$); IR ($CHCl_3$) 2950, 2130, 1445, 1380 cm^{-1}; ^1H NMR ($CDCl_3$) 1.34 (s, 3 H), 1.63 (s, 3 H), 1.65 (s, 3 H), 1.69 (s, 3 H), 5.09 (br t, 1 H, J = 7 Hz), 5.38 (br s, 1 H); ^{13}C NMR ($CDCl_3$) 133.8 (s), 132.5 (s), 129.7 (br s), 122.8 (d), 119.8 (d), 66.8 (s), 43.0 (d), 39.0 (t), 30.6 (t), 26.6 (t), 25.7 (q), 24.0 (t), 23.4 (q), 23.2 (q), 22.7 (q), 17.6 (q); HRMS, obsd m/z 263.1707, $C_{16}H_{25}NS$ requires m/z 263.1708.

Guaia-1(5),6-diene (7): oil; $[\alpha]^{20}_D$ −30.2° (*c* 2.5, $CHCl_3$); IR (CCl_4) 2940, 1450 cm^{-1}; UV ($CHCl_3$) 268 nm (ε 6700); ^1H NMR ($CDCl_3$) δ 1.01 (d, 3 H, J = 7 Hz), 1.02 (d, 6 H, J = 7 Hz), 1.03 (d, 3 H, J = 7 Hz), 1.35 (m, 1 H), 1.67 (m, 1 H), 1.72 (m, 1 H), 1.97 (m, 1 H), 2.1–2.2 (3 H), 2.31 (m, 1 H), 2.46 (m, 1 H), 2.62 (m, 2 H), 5.52 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 151.1 (s), 142.2 (s), 135.9 (s), 117.1 (d), 45.0 (d), 37.7 (d), 36.5 (d), 35.8 (t), 33.6 (t), 31.0 (t), 27.6 (t), 21.5 (q), 21.5 (q), 20.7 (q), 20.2 (q); HRMS, obsd m/z 204.1877, $C_{15}H_{24}$ requires 204.1878.

(6*R*,7*E*)-α-Bisabolene. Methyl iodide (excess) and potassium carbonate (excess) were added to a solution of the amine **3** (395 mg, 1.8 mmol) in acetone (15 mL), and the mixture was boiled under reflux for 8 h. The cooled solution was poured into water (30 mL), and the product was extracted with ethyl acetate (3 × 20 mL). The combined extracts were dried over sodium sulfate and filtered, and the solvent was evaporated to obtain a clear oil (251 mg). The product was a mixture of bisabolene isomers that was separated by flash chromatography on 25% $AgNO_3$/silica gel. (6*R*,7*E*)-α-Bisabolene (115 mg, 32% theoretical) was eluted with 2% ether/hexane: $[\alpha]_D$ +33° (*c* 0.43, $CHCl_3$); ^1H NMR (CCl_4) δ 1.59 (s, 3 H), 1.60 (s, 3 H), 1.61 (s, 3 H), 1.66 (s, 3 H), 2.26 (m, 1 H), 2.62 (t, 2 H, J = 7 Hz), 5.04 (br t, 1 H, J = 7 Hz), 5.06 (br t, 1 H, J = 7 Hz), 5.32 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 139.3 (s), 133.6 (s), 131.2 (s), 123.6 (d), 121.9 (d), 121.0 (d), 42.9 (d), 30.9 (t, t) 28.1 (t), 27.0 (t), 25.6 (q), 23.4 (q), 17.7 (q), 14.2 (q); MS, m/z 204.

Conversion of Amine 3 into Urea 5. Carbonyldiimidazole (40 mg, 0.25 mmol) was added to a solution of the amine (45 mg, 0.20 mmol) in THF (10 mL) and the mixture was stirred at 25 °C for 16 h. The product contained a mixture of the urea and unreacted amine. The product was separated by LC on Partisil using 20% ether in hexane as eluant to obtain a sample (12 mg, 24% theoretical) of the urea **5**, $[\alpha]_D$ +45° (*c* 1.2, $CHCl_3$), that had spectral data identical with those of the natural product.

Single-Crystal X-ray Diffraction Analysis of Urea 5. Preliminary X-ray photographs displayed tetragonal symmetry. Accurate lattice parameters of $a = b = 18.306$ (4) and $c = 9.611$ (2) Å were determined from a least-squares fit of 15 diffractometer-measured 2θ values. The systematic extinctions, optical rotation, and crystal density were uniquely accommodated by space group $P4_2$ with four molecules of composition $C_{31}H_{52}N_2O$ comprising the unit cell. All unique diffraction maxima with $2\theta \leq 114°$ were measured on a computer-controlled four-circle diffractometer using variable speed, 1° ω-scans and graphite-monochromated Cu K$\bar{\alpha}$ radiation (1.54178 Å). Of the 2328 reflections surveyed in this fashion, only 756 (33%) were judged observed after correction for Lorentz, polarization, and background effects ($|F_o| \geq 3\sigma(F_o)$). A phasing model was found with some difficulty, and the use of the program DIRDIF for phase recycling was crucial.[3] Block-diagonal least-squares refinements with anisotropic non-hydrogen atoms and calculated hydrogens have converged to a standard crystallographic residual of 0.078 for the observed reflections. Additional crystallographic information is available and is described in the paragraph entitled Supplementary Material Available at the end of this paper.

Dehydrogenation of Guaia-1(5),6-diene (7). Palladium on carbon (10%) catalyst (~5 mg) was added to a solution of the diene **7** (12 mg, 0.06 mmol) in xylene (5 mL) and the mixture was boiled under reflux for 36 h. The product was filtered, the solvent was evaporated, and the residue was chromatographed on silica gel to obtain guaiazulene, identical in all respects with an authentic sample.

Oxidation of Guaia-1(5),6-diene (7) with Singlet Oxygen. A solution of the diene **7** (90 mg, 0.44 mmol) and rose bengal (2 mg) in 95:5 dichloromethane–methanol (20 mg) was irradiated with a 200-W incandescent lamp under an atmosphere of oxygen for 6 h. The reaction mixture was evaporated and redissolved in ether, and the solution was filtered through a plug of silica gel to remove the catalyst. The crude product (100 mg) was separated by LC on μ-Partisil using 15% ether in hexane as eluant to obtain two isomeric peroxides, **8a** (11 mg, 10% theoretical) and **8b** (23 mg, 20% theoretical).

Peroxide 8a: oil; ^{1}H NMR ($CDCl_3$) δ 0.99 (d, 6 H, J = 7 Hz), 1.06 (d, 3 H, J = 7 Hz), 1.19 (d, 3 H, J = 7 Hz), 2.65 (m, 1 H), 5.72 (d, 1 H, J = 2.3 Hz).

Peroxide 8b: oil; ^{1}H NMR ($CDCl_3$) δ 0.92 (d, 3 H, J = 7 Hz), 0.96 (d, 3 H, J = 7 Hz), 0.97 (d, 3 H, J = 7 Hz), 1.13 (d, 3 H, J = 7 Hz), 2.87 (m, 1 H), 6.0 (d, 1 H, J = 2.2 Hz).

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Registry No. **3**, 105281-43-4; **4**, 105281-44-5; **5**, 105281-45-6; **6**, 105281-46-7; **7**, 105369-35-5; **8**, 105281-47-8; (6*R*,7*E*)-α-bisabolene, 70286-31-6; guaiazulene, 489-84-9.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond distances, and bond angles (6 pages). Ordering information is given on any current masthead page.

(3) All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: REDUCE and UNIQUE, data programs by M. E. Leonowicz, Cornell University, 1978; MULTAN 78, MULTAN 80, and RANTAN 80, systems of computer programs for the automatic solution of crystal structures from X-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, University of York, England, 1978 and 1980; DIRDIF written by P. T. Buerskens et al., University of Nijmegan, Netherlands, 1981; BLS78A, an anisotropic block-diagonal least-squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1978, and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.

Nitrogenous Bisabolene Sesquiterpenes from Marine Invertebrates

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Three bisabolene sesquiterpenes, an amine hydrochloride (**8**), an isocyanate (**9**), and an isocyano derivative (**16**), were isolated from a sponge, *Ciocalypta* sp. The structure of the isocyanate was proven by X-ray diffraction of its (*p*-bromobenzyl)urea derivative. From a nudibranch, *Phyllidia* sp., from Sri Lanka, we isolated the previously unreported 3-isocyanotheonellin (**11**).

Bisabolene sesquiterpenes are widely distributed in nature. A notable terrestrial representative is hernandulcin, the sweet principle of *Lippia dulcis* (Verbenaceae).[2] Marine bisabolenes include an alcohol from a gorgonian,[3] 3-isothiocyanato- (**1**) and 3-formamidotheonellin (**2**) from a sponge, *Theonella* cf. *swinhoei*,[4] and four nitrogenous derivatives from a sponge, *Halichondria* sp., which were isolated in Faulkner's laboratory and are described in the accompanying paper.[5] The *Halichondria* metabolites are a 7(*S*)-amine (**3**) and its hydrochloride salt (**4**), a 7(*S*)-isothiocyanate (**5**), and a urea bisdihydrobisabolene (**6**).

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