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Metabolites from the Marine Sponge *Epipolasis kushimotoensis*

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Two isothiocyanates, epipolasin-A **1** and -B **2**, and two corresponding thiourea derivatives, epipolasinthiourea-A **14** and -B **15**, were isolated from the marine sponge *E. kushimotoensis*. Their structures were elucidated on the basis of chemical and spectral evidence. The thioureas **14** and **15** showed cell growth inhibition activity.

Keywords——sponge; *Epipolasis kushimotoensis*; epipolasin-A, -B; epipolasinthiourea-A, -B; sesquiterpene; isothiocyanate; thiourea derivative; cell growth inhibition

Sesquiterpene isothiocyanates, which are rather rare in terrestrial plants, have been isolated from marine sponges such as *Halichondria* sp.[1] and *Axinella cannabina*.[2-5] Our continuing search for bioactive metabolites from Porifera yielded five sesquiterpene isothiocyanates, epipolasin-A through -E, from *E. kushimotoensis*, together with two thiourea derivatives, epipolasinthiourea-A and-B, which are adducts of β-phenethylamine with epipolasin-A (**1**) and -B (**2**), respectively.

Chromatography of the dichloromethane extract obtained by direct immersion of the frozen material gave fatty acids, glycerin monoalkylether, and $\Delta^{5,7}$-sterols as known compounds, as well as sesquiterpene derivatives.

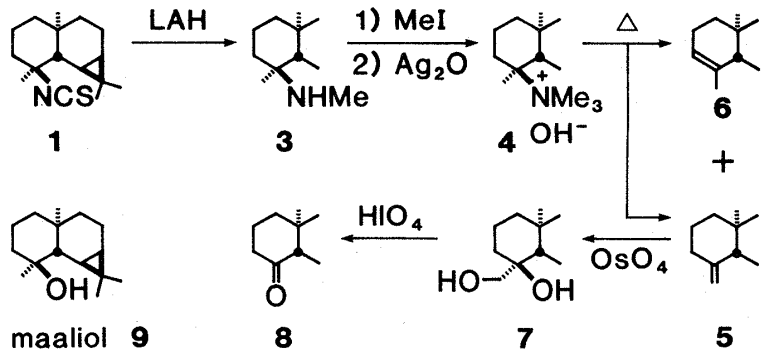


Chart 1

Epipolasin-A (**1**): mp 49—50 °C, $[\alpha]_D$ +7.6±0.5° (c=1.0, $CHCl_3$). Infrared spectra (IR) (2100 cm^{-1}, –NCS), proton nuclear magnetic resonance (^{1}H-NMR) [δ, 0.55, 0.67 (cyclopropane), 0.88, 1.01, 1.14, and 1.44 ppm (s, Me×4)], mass spectra (MS) (M^+ 263), and elementary analysis ($C_{16}H_{25}NS$) indicated **1** to be a tricyclic sesquiterpene isothiocyanate. Compound **1** was then subjected to a series of degradation reactions, which are outlined in Chart 1. $LiAlH_4$ reduction of the isothiocyanate group to a secondary amine **3** followed by quaternization, anion exchange, and Hofmann degradation gave the olefins **5** and **6**. The *exo*-methylene compound **5** was led to a diol **7**, which was in turn converted to the norketone **8**. The IR (1708 cm^{-1}) and circular dichroism (CD) ($[\theta]_{287}$ +4180) spectra suggested a six-

membered cyclic ketone and the absolute configuration of the decalone system shown in **8**. Direct comparison of the *exo*-methylene compound **5**, the diol **7**, and the norketone **8** derived from **1** proved them to be identical with those derived from natural maaliol (**9**),[6] including their absolute configurations. The configuration of the NCS group was determined as β-equatorial from the fact that the Hofmann degradation products **5** and **6** were obtained in a ratio of about 3:1. On the basis of the chemical and spectral results mentioned above, epipolasin-A can be represented as **1**.

Although this formula is the same as that of an isothiocyanate isolated from the nudibranch *Cadlina luteomarginata*,[7] the $[\alpha]_D$ values are different (in ref. 7: $-12°$, $c=1.1$, $CHCl_3$; $+7.6°$ for **1**. The structure elucidation of the isothiocyanate in ref. 7 was based on the analogy with the corresponding formamide (–NHCHO instead of –NCS), which was deduced from the results of an X-ray analysis that was not conclusive, as noted in a footnote of ref. 7. Therefore, **1** probably differs from the isothiocyanate in ref. 7 with regard to stereochemistry.

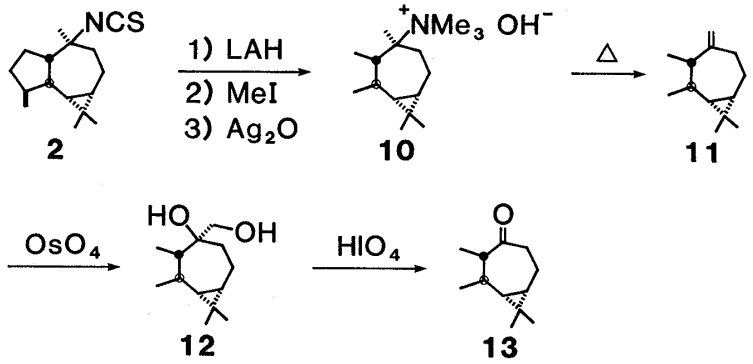
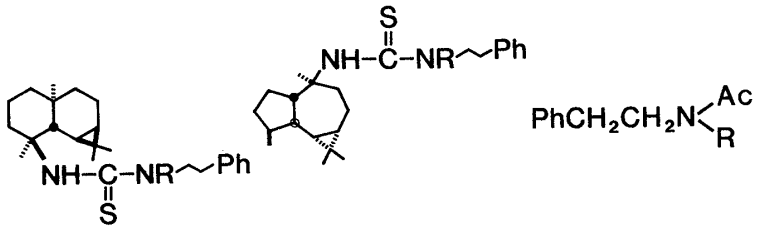


Chart 2

Epipolasin-B (**2**): mp 92 °C, $[\alpha]_D$ $+91.2\pm1.3°$ ($c=1.0$, $CHCl_3$), IR (2100 cm^{-1}, –NCS), ^{1}H-NMR [δ, 0.5—0.7 (cyclopropane), 0.92 (*sec*-Me), 0.97, 1.01, and 1.28 ppm (s, Me×3)], MS (M^+ 263), and elementary analysis ($C_{16}H_{25}NS$) also suggested **2** to be a tricyclic sesquiterpene isothiocyanate. The degradation reactions performed on **1** were also applied to epipolasin-B (Chart 2), and gave the norketone **13** *via* the *exo*-methylene compound **11**. The derived *exo*-methylene compound **11** and norketone **13** were found to be identical with the known compounds (−)-aromadendrene[8] and (+)-apoaromadendrone,[8] respectively (mp, $[\alpha]_D$, IR and ^{1}H-NMR).

On the basis of the above-mentioned results, the structure of epipolasin-B can be represented as **2**, including the absolute configuration. The same structure without the stereochemistry has been reported for axisothiocyanate-2,[3] although the $[\alpha]_D$ values differ greatly ($+12.8°$ for axisothiocyanate-2, $+76.7°$ for **2**). Therefore, **2** must be different from axisothiocyanate-2 with regard to stereochemistry.



14 : R=H **15** : R=H **18** : R=H
16 : R=Ac **17** : R=Ac **19** : R=Ac

Chart 3

From the more polar fraction, two other components, epipolasinthiourea-A (**14**) and -B (**15**), were isolated and were found to be closely analogous to **1** and **2**, respectively. They were thought to be thiourea derivatives from their IR [3400 (NH), *ca.* 1500 cm^{-1} (>C=S)] and ^{1}H-NMR (signals of cyclopropane, Me's, and phenethyl moiety) spectra. These spectral data together with the MS (M^+ 384) suggested that they were phenethylurea derivatives. Acetylation of **14** and **15** at room temperature afforded monoacetyl derivatives **16** and **17**, respectively, whereas acetylation at 90 °C gave **1** and **2**, respectively, together with the mono- and diacetylphenethylamine **18** and **19**, leading to the structures of **14** for epipolasinthiourea-A and **15** for -B.

Three other sesquiterpene isothiocyanates, epipolasin-C, -D, and -E, were also isolated as minor components, and elucidation of their structures is in progress.

The thiourea derivatives **14** and **15** showed moderate cytotoxic activities *in vitro* [L1210 cells; ED_{50}, 4.1 μg/ml for **14** and 3.7 μg/ml for **15**], but the other components, epipolasin-A through -E, did not show any significant bioactivities (*e.g.* antibacterial, antiviral, and cytotoxic activities *in vitro*).

Experimental

Isolation——The frozen sponge material (dry weight 94 g after extraction) was directly immersed in dichloromethane for simultaneous defrosting and extraction. The resulting extract (5.1 g) was chromatographed on silica gel (15 g, eluted with *n*-hexane, CH_2Cl_2, and 10% MeOH–CH_2Cl_2 succesively) for group separation (fr. 1 to fr. 5). Separation of the non-polar fraction (fr. 1, 2.1 g) on a Lobar B column (eluted with *n*-hexane) gave the sesquiterpene isothiocyanate fraction (1.47 g). Preparative RP-high performance liquid chromatography (HPLC) [Nucleosil 30C_{18} packed in a GCH[9] column (i.d. 20 × 250 mm)/repeated sample application of 200 mg/90% MeOH–H_2O as an eluant] afforded epipolasin-C (129 mg), -A (935 mg), -B (268 mg), -E (4 mg), and -D (40 mg). All components showed the –NCS band (ν, 2100 cm^{-1}) and gave *m/z* 263 as M^+, indicative of sesquiterpene isothiocyanate.

Epipolasin-A (**1**): UV $\lambda_{max}^{ethanol}$ nm (ε): 247 (1400). *Anal.* Calcd for $C_{16}H_{25}NS$: C, 72.95; H, 9.53; N, 5.32; S, 12.16. Found: C, 72.80; H, 9.42; N, 5.38; S, 12.10.

Epipolasin-B (**2**): UV $\lambda_{max}^{ethanol}$ nm (ε): 248 (1600). *Anal.* Calcd for $C_{16}H_{25}NS$: C, 72.95; H, 9.53; N, 5.32; S, 12.16. Found: C, 72.93; H, 9.58; N, 5.37; S, 12.01.

Epipolasin-C: Oil; $[\alpha]_D$ −67.9 ± 1.1 ° (*c*=1.0, $CHCl_3$). UV $\lambda_{max}^{ethanol}$ nm (ε): 244 (1100). IR ($CHCl_3$): 2125 cm^{-1} (NCS). ^{1}H-NMR ($CDCl_3$) δ: 0.6—0.9 (cyclopropane), 1.02, 1.03 (d, *sec*-Me × 2), 1.00, 1.07 ppm (s, Me × 2).

Epipolasin-D: Oil; $[\alpha]_D$ +66.0 ± 1.1 ° (*c*=1.0, $CHCl_3$). UV $\lambda_{max}^{ethanol}$ nm (ε): 247 (1500). IR ($CHCl_3$): 2120 (NCS), 1675 cm^{-1} (olefin). ^{1}H-NMR ($CDCl_3$) δ: 0.75, 0.95 (d, *sec*-Me × 2), 1.28 (s, Me), 1.67 (br s, olefin-Me), 5.47 ppm (1H, br s, olefin-H).

Epipolasin-E: Oil; IR ($CHCl_3$): 2200 cm^{-1} (NCS).

The thiourea fraction (228 mg) was obtained from fr. 3 (1.1 g), which was detectable by UV light on the thin layer chromatography (TLC) plate, by using a Lobar B column (eluted with 2% MeCN–CH_2Cl_2). Further separation of this fraction [LiChrosorb 10 μm in a i.d. 8 × 250 mm stainless steel column/hexane–$CHCl_3$–AcOEt (8 : 1 : 1)] furnished two components, which were purified by preparative TLC.

Epipolasinthiourea-A (**14**): 41 mg; oil, IR ($CHCl_3$): 3400 (NH), 1495 cm^{-1} (>C=S). ^{1}H-NMR ($CDCl_3$) δ: 0.3—0.8 (cyclopropane), 0.87, 0.93, 1.07, 1.30 (s, Me × 4), 2.93 (t, Ph-CH_2), 3.82 (dt, NH–CH_2), 7.28 ppm (Ph). MS *m/z*: 384 (M^+).

Epipolasinthiourea-B (**15**): 41 mg; oil, IR ($CHCl_3$): 3410 (NH), 1500 cm^{-1} (>C=S). ^{1}H-NMR ($CDCl_3$) δ: 0.3—0.6 (cyclopropane), 0.85 (d, *sec*-Me), 0.93, 0.98, 1.08 (s, Me × 3), 2.95 (t, Ph–CH_2), 3.95 (dt, NH–CH_2), 7.30 ppm (Ph). MS *m/z*: 384 (M^+).

Fatty acids, $\Delta^{5,7}$-sterols, and glycerin monoalkylethers were separated from the polar fractions (fr. 4 and fr. 5, 1.5 g) by using a Lobar column B and were identified from the IR and ^{1}H-NMR data.

$LiAlH_4$ Reduction of **1**——To a solution of **1** (202 mg) in ether (5 ml), $LiAlH_4$ (181 mg) was added and the mixture was stirred for 1 h at room temperature. Work-up as usual gave an oily amine (**3**; 178 mg, 99%). IR ($CHCl_3$): 3150 cm^{-1} (NH). ^{1}H-NMR ($CDCl_3$) δ: 0.4—0.7 (cyclopropane), 0.92, 0.95, 1.02, 1.08 (s, Me × 4), 2.27 ppm (NHMe). MS *m/z*: 235 (M^+).

HCl Salt: mp 250—265 °C (dec.), $[\alpha]_D$ +3.8 ° (*c*=0.8, $CHCl_3$).

N-Acetyl: mp 154—155 °C, $[\alpha]_D$ +56.0 ° (*c*=1.0, $CHCl_3$). IR ($CHCl_3$): 1640 cm^{-1} (NAc). ^{1}H-NMR ($CDCl_3$) δ: 2.10 (NAc), 2.97 ppm (NMe). MS *m/z*: 277 (M^+).

Methohydroxide (**4**)——A solution of the amine **3** (80 mg), MeI (1 ml), and K_2CO_3 (81 mg) in EtOH (2 ml) was

refluxed for 1 h. Evaporation of the solvent and trituration of the residue with ether gave the methiodide (128 mg, 96%, mp 232—248 °C dec.), which was treated with freshly prepared wet Ag_2O in MeOH (5 ml). The mixture was stirred for 30 min at room temperature, then evaporation of the MeOH and trituration of the residue with ether gave the methohydroxide **4** (67 mg, 68%) and an olefin (23 mg, 32%), which was identified as the *exo*-methylene compound **5** described below.

Hofmann Degradation of 4——The methohydroxide **4** (159 mg) was heated for 20 min at 145 °C under an Ar atmosphere. The product was found to be a mixture of olefins **5** and **6** by ^{1}H-NMR and to be a 3 : 1 mixture by HPLC analysis. The mixture was separated into the two components by preparative RP-HPLC [Develosil ODS 10—20 μm (Nomura Kagaku, Seto, Japan) packed in a GCH column (i.d. 25 × 250 mm)/85% MeOH–H_2O].

exo-Olefin (**5**): Oil (92 mg); $[\alpha]_D$ $-15.2 \pm 0.5°$ ($c=1.0$, $CHCl_3$). IR ($CHCl_3$): 3075, 1641, 890 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.6—0.8 (cyclopropane), 0.74, 0.94, 1.04 (s, Me × 3), 4.80, 4.84 ppm (=CH_2).

endo-Olefin (**6**): Oil (31 mg); IR ($CHCl_3$): 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.6—0.8 (cyclopropane), 0.83, 0.90, 1.03 (s, Me × 3), 1.42 (olefin-Me), 5.35 ppm (olefin-H).

OsO_4 Oxidation of 5——A solution of OsO_4 (271 mg) in ether (3 ml) and pyridine (170 mg) was added to a solution of **5** (187 mg) in ether (2.7 ml), and the mixture was left to stand for 21 h at 3 °C. The black precipitate (osmate–pyridine complex, 372 mg) was dissolved in 50% aq. MeOH (9 ml) containing $NaHSO_3$ (630 mg) and the solution was refluxed for 1 h. Filtration, dilution of the filtrate with water, then extraction with ether furnished the crude diol (135 mg, mp 136—138 °C), which was recrystallized from *n*-hexane, yielding pure **7**: mp 142 °C; $[\alpha]_D$ $+28.9 \pm 0.7°$ ($c=1.0$, $CHCl_3$). IR ($CHCl_3$): 3580, 3440 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.5—0.8 (cyclopropane), 0.82, 0.95, 1.05 (s, Me × 3), 3.71 ppm (s, CH_2OH).

Norketone (8)——A mixture of a solution of the diol **7** (36 mg) in dioxane (1 ml), and a solution of $NaIO_4$ (40 mg) in H_2O (1 ml) was stirred for 1 h at room temperature. This mixture was poured into water, and extraction with ether gave the norketone **8** (oil, 35 mg); $[\alpha]_D$ $+54.4 \pm 0.9°$ ($c=1.1$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.6—0.8 (cyclopropane), 0.77, 0.88, 1.03 ppm (s, Me × 3).

Degradation of 2——The reaction conditions were as described above.

a) Treatment of **2** (98 mg) with $LiAlH_4$ yielded the oily amine (86 mg, 98%). IR ($CHCl_3$): 3100 cm^{-1} (NH). ^{1}H-NMR ($CDCl_3$) δ: 0.4—0.6 (cyclopropane), 0.92 (d, *sec*-Me), 0.92, 1.00, 1.00 (s, Me × 3) 2.30 ppm (NHMe).

b) The amine obtained (73 mg) was quaternized to give the methiodide (99 mg), which was in turn led to the methohydroxide **10** (71 mg, 80%).

c) Hofmann reaction of **10** (70 mg) exclusively gave the oily **11**: $[\alpha]_D$ $-3.2 \pm 0.4°$ ($c=1.1$, $CHCl_3$). IR ($CHCl_3$): 1637, 895 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.5—0.7 (cyclopropane), 0.96 (d, *sec*-Me), 0.97, 1.03 (s, Me × 2) 4.61 ppm (=CH_2).

d) Hydroxylation of **11** (44 mg) with OsO_4 (66 mg) gave the crude diol (51 mg), which was purified by preparative HPLC (LiChrosorb SI-60, 10 μm/i.d. 10 × 250 mm, stainless steel column/5% MeCN–CH_2Cl_2) followed by recrystallization from *n*-hexane, to obtain pure **12**: mp 118 °C; $[\alpha]_D$ $+40.9 \pm 0.8°$ ($c=1.0$, $CHCl_3$). IR ($CHCl_3$) : 3600, 3440 cm^{-1}. ^{1}H-NMR ($CDCl_3 + D_2O$) δ: 0.5—0.7 (cyclopropane), 0.91 (d, *sec*-Me), 0.97, 1.01 (s, Me × 2), 3.60 ppm (s, $-CH_2OD$).

e) Cleavage of **12** (42 mg) with $NaIO_4$ (58 mg) afforded the norketone **13** (34 mg): mp 83—84 °C; $[\alpha]_D$ $+3.3 \pm 0.4°$ ($c=1.0$, $CHCl_3$). CD (methanol) [θ] (nm): +2280 (280). IR ($CHCl_3$): 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.6—0.9 (cyclopropane), 0.84 (d, *sec*-Me), 0.93, 1.08 ppm (s, Me × 2).

Acetylation of 14——a) Acetylation of **14** (23 mg) with Ac_2O–pyridine at room temperature gave a monoacetyl derivative **16** (oil, 23 mg): IR ($CHCl_3$): 3450 (NH), 1670 cm^{-1} (NAc). ^{1}H-NMR ($CDCl_3$) δ: 0.6—0.9 (cyclopropane), 0.96, 1.00, 1.02, 1.68 (s, Me × 4), 2.22 (s, NAc), 3.00 (t, $PhCH_2$), 4.40 (t, N–CH_2), 7.28 ppm (Ph). MS *m/z*: 426 (M^+), $C_{26}H_{38}N_2OS$.

b) A solution of **14** (18 mg) in Ac_2O (0.3 ml) was heated at 90 °C. After evaporation of Ac_2O under a stream of nitrogen, the residue was subjected to preparative RP-HPLC (Nucleosil 7C_{18}/GCH column, i.d. 10 × 250 mm/MeOH) to yield **1** (10 mg) and a mixture of mono- and diacetylphenetylamine **18** and **19** (7 mg).

Acetylation of 15——Acetylation of **15** (17 mg) at room temperature gave a monoacetyl derivative **17** (oil, 11 mg): IR ($CHCl_3$): 2320 (NH), 1660 cm^{-1} (NAc). ^{1}H-NMR ($CDCl_3$) δ: 0.5—0.7 (cyclopropane), 0.92 (d, *sec*-Me), 0.98, 1.01, 1.40 (s, Me × 3), 2.22 (NAc), 3.02 (t, $PhCH_2$), 4.37 (t, N–CH_2), 7.28 ppm (Ph). MS *m/z*: 426 (M^+), $C_{26}H_{38}N_2OS$. Acetylation of **15** (11 mg) at 90 °C afforded **2** (5 mg) and a mixture of mono- and diacetylphenetylamine **18** and **19** (3 mg).

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9) A glass column (o.d. 28 mm, i.d. 20 mm) protected by an external brass cylinder with windows, manufactured by Umetani Seiki, Osaka, Japan.