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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, $[\alpha]_D +7.6 \pm 0.5^\circ$ ($c=1.0$, CHCl₃). Infrared spectra (IR) (2100 cm⁻¹, -NCS), proton nuclear magnetic resonance (¹H-NMR) [δ , 0.55, 0.67 (cyclopropane), 0.88, 1.01, 1.14, and 1.44 ppm (s, Me $\times$ 4)], mass spectra (MS) (M^+ 263), and elementary analysis (C₁₆H₂₅NS) 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. LiAlH₄ 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 ; $+7.6^\circ$ for **1**). The structure elucidation of the isothiocyanate in ref. 7 was based on the analogy with the corresponding formamide ($-\text{NHCHO}$ instead of $-\text{NCS}$), 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 , $[\alpha]_D +91.2 \pm 1.3^\circ$ ($c=1.0$, CHCl_3), IR (2100 cm^{-1} , $-\text{NCS}$), $^1\text{H-NMR}$ [δ , 0.5–0.7 (cyclopropane), 0.92 (*sec*-Me), 0.97, 1.01, and 1.28 ppm (s, $\text{Me} \times 3$)], MS (M^+ 263), and elementary analysis ($\text{C}_{16}\text{H}_{25}\text{NS}$) 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 (–)-aromadendrene⁸) and (+)-apoaromadendrone,⁸) respectively (mp, $[\alpha]_D$, IR and $^1\text{H-NMR}$).

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 axisothiocyante-2,³) although the $[\alpha]_D$ values differ greatly ($+12.8^\circ$ for axisothiocyante-2, $+76.7^\circ$ for **2**). Therefore, **2** must be different from axisothiocyante-2 with regard to stereochemistry.

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} ($>\text{C}=\text{S}$)] and ^1H -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 $\mu\text{g}/\text{ml}$ for **14** and 3.7 $\mu\text{g}/\text{ml}$ 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% $\text{MeOH}-\text{CH}_2\text{Cl}_2$ successively) 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 30C₁₈ packed in a GCH⁹ column (i.d. 20 $\times$ 250 mm)/repeated sample application of 200 mg/90% $\text{MeOH}-\text{H}_2\text{O}$ 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 $\lambda_{\text{max}}^{\text{ethanol}}$ nm (ϵ): 247 (1400). *Anal.* Calcd for $\text{C}_{16}\text{H}_{25}\text{NS}$: 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 $\lambda_{\text{max}}^{\text{ethanol}}$ nm (ϵ): 248 (1600). *Anal.* Calcd for $\text{C}_{16}\text{H}_{25}\text{NS}$: 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; $[\alpha]_{\text{D}} -67.9 \pm 1.1^\circ$ ($c=1.0$, CHCl_3). UV $\lambda_{\text{max}}^{\text{ethanol}}$ nm (ϵ): 244 (1100). IR (CHCl_3): 2125 cm^{-1} (NCS). ^1H -NMR (CDCl_3) δ : 0.6–0.9 (cyclopropane), 1.02, 1.03 (d, *sec*-Me $\times$ 2), 1.00, 1.07 ppm (s, Me $\times$ 2).

Epipolasin-D: Oil; $[\alpha]_{\text{D}} +66.0 \pm 1.1^\circ$ ($c=1.0$, CHCl_3). UV $\lambda_{\text{max}}^{\text{ethanol}}$ nm (ϵ): 247 (1500). IR (CHCl_3): 2120 (NCS), 1675 cm^{-1} (olefin). ^1H -NMR (CDCl_3) δ : 0.75, 0.95 (d, *sec*-Me $\times$ 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% $\text{MeCN}-\text{CH}_2\text{Cl}_2$). Further separation of this fraction [LiChrosorb 10 μm in a i.d. 8 $\times$ 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} ($>\text{C}=\text{S}$). ^1H -NMR (CDCl_3) δ : 0.3–0.8 (cyclopropane), 0.87, 0.93, 1.07, 1.30 (s, Me $\times$ 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} ($>\text{C}=\text{S}$). ^1H -NMR (CDCl_3) δ : 0.3–0.6 (cyclopropane), 0.85 (d, *sec*-Me), 0.93, 0.98, 1.08 (s, Me $\times$ 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 ^1H -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). ^1H -NMR (CDCl_3) δ : 0.4–0.7 (cyclopropane), 0.92, 0.95, 1.02, 1.08 (s, Me $\times$ 4), 2.27 ppm (NHMe). MS m/z : 235 (M^+).

HCl Salt: mp 250–265 °C (dec.), $[\alpha]_{\text{D}} +3.8^\circ$ ($c=0.8$, CHCl_3).

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

Methoxyhydroxide (**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 Ag₂O 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 ¹H-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–H₂O].

exo-Olefin (**5**): Oil (92 mg); $[\alpha]_D -15.2 \pm 0.5^\circ$ ($c=1.0$, CHCl₃). IR (CHCl₃): 3075, 1641, 890 cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.6–0.8 (cyclopropane), 0.74, 0.94, 1.04 (s, Me × 3), 4.80, 4.84 ppm (=CH₂).

endo-Olefin (**6**): Oil (31 mg); IR (CHCl₃): 1600 cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.6–0.8 (cyclopropane), 0.83, 0.90, 1.03 (s, Me × 3), 1.42 (olefin-Me), 5.35 ppm (olefin-H).

OsO₄ Oxidation of 5—A solution of OsO₄ (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 NaHSO₃ (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; $[\alpha]_D +28.9 \pm 0.7^\circ$ ($c=1.0$, CHCl₃). IR (CHCl₃): 3580, 3440 cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.5–0.8 (cyclopropane), 0.82, 0.95, 1.05 (s, Me × 3), 3.71 ppm (s, CH₂OH).

Norketone (8)—A mixture of a solution of the diol **7** (36 mg) in dioxane (1 ml), and a solution of NaIO₄ (40 mg) in H₂O (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); $[\alpha]_D +54.4 \pm 0.9^\circ$ ($c=1.1$, CHCl₃). ¹H-NMR (CDCl₃) 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 LiAlH₄ yielded the oily amine (86 mg, 98%). IR (CHCl₃): 3100 cm⁻¹ (NH). ¹H-NMR (CDCl₃) 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**: $[\alpha]_D -3.2 \pm 0.4^\circ$ ($c=1.1$, CHCl₃). IR (CHCl₃): 1637, 895 cm⁻¹. ¹H-NMR (CDCl₃) δ : 0.5–0.7 (cyclopropane), 0.96 (d, *sec*-Me), 0.97, 1.03 (s, Me × 2) 4.61 ppm (=CH₂).

d) Hydroxylation of **11** (44 mg) with OsO₄ (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–CH₂Cl₂) followed by recrystallization from *n*-hexane, to obtain pure **12**: mp 118 °C; $[\alpha]_D +40.9 \pm 0.8^\circ$ ($c=1.0$, CHCl₃). IR (CHCl₃): 3600, 3440 cm⁻¹. ¹H-NMR (CDCl₃ + D₂O) δ : 0.5–0.7 (cyclopropane), 0.91 (d, *sec*-Me), 0.97, 1.01 (s, Me × 2), 3.60 ppm (s, –CH₂OD).

e) Cleavage of **12** (42 mg) with NaIO₄ (58 mg) afforded the norketone **13** (34 mg): mp 83–84 °C; $[\alpha]_D +3.3 \pm 0.4^\circ$ ($c=1.0$, CHCl₃). CD (methanol) $[\theta]$ (nm): +2280 (280). IR (CHCl₃): 1690 cm⁻¹. ¹H-NMR (CDCl₃) 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 Ac₂O–pyridine at room temperature gave a monoacetyl derivative **16** (oil, 23 mg): IR (CHCl₃): 3450 (NH), 1670 cm⁻¹ (NAc). ¹H-NMR (CDCl₃) δ : 0.6–0.9 (cyclopropane), 0.96, 1.00, 1.02, 1.68 (s, Me × 4), 2.22 (s, NAc), 3.00 (t, PhCH₂), 4.40 (t, N–CH₂), 7.28 ppm (Ph). MS m/z : 426 (M⁺), C₂₆H₃₈N₂OS.

b) A solution of **14** (18 mg) in Ac₂O (0.3 ml) was heated at 90 °C. After evaporation of Ac₂O under a stream of nitrogen, the residue was subjected to preparative RP-HPLC (Nucleosil 7C₁₈/GCH column, i.d. 10 × 250 mm/MeOH) to yield **1** (10 mg) and a mixture of mono- and diacetylphenethylamine **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 (CHCl₃): 2320 (NH), 1660 cm⁻¹ (NAc). ¹H-NMR (CDCl₃) 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, PhCH₂), 4.37 (t, N–CH₂), 7.28 ppm (Ph). MS m/z : 426 (M⁺), C₂₆H₃₈N₂OS. Acetylation of **15** (11 mg) at 90 °C afforded **2** (5 mg) and a mixture of mono- and diacetylphenethylamine **18** and **19** (3 mg).

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References

- 1) B. J. Bureson, C. Christophersen, and P. J. Scheuer, *Tetrahedron*, **31**, 2015 (1975).
- 2) F. Cafieri, E. Fattorusso, S. Magno, C. Santacroce, and D. Sica, *Tetrahedron*, **29**, 4259 (1973); *idem, ibid.*, **30**, 3911 (1974).

- 3) E. Fattorusso, S. Magno, L. Mayol, C. Santacroce, and D. Sica, *Tetrahedron*, **31**, 269 (1975).
- 4) B. Di Blasio, E. Fattorusso, S. Magno, L. Mayol, C. Pedone, C. Santacroce, and D. Sica, *Tetrahedron*, **32**, 473 (1976).
- 5) A. Iengo, L. Mayol, and C. Santacroce, *Experientia*, **33**, 11 (1977).
- 6) R. B. Bates, G. Büchi, T. Matsuura, and R. R. Schaffer, *J. Am. Chem. Soc.*, **82**, 2327 (1960).
- 7) J. E. Thompson, R. P. Walker, S. J. Wratten, and D. J. Faulkner, *Tetrahedron*, **38**, 1865 (1982).
- 8) G. Büchi, W. Hofheinz, and J. V. Paukstelis, *J. Am. Chem. Soc.*, **91**, 6473 (1969).
- 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.