

NOVEL BISABOLENE-TYPE SESQUITERPENOID WITH A CONJUGATED DIENE  
ISOLATED FROM THE OKINAWAN SEA SPONGE *THEONELLA* CF. *SWINHOEI*<sup>1)</sup>

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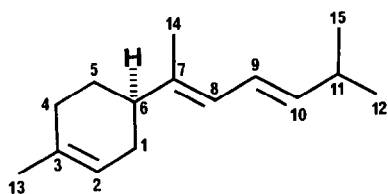
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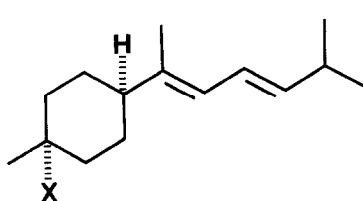
Summary: Three novel sesquiterpenoids have been isolated from the Okinawan sea sponge *Theonella* cf. *swinhoei* and the structures have been determined on the basis of the spectral data and chemical reactions.

Numerous bioactive marine natural products having rare functional groups have been obtained from sea sponges.<sup>2)</sup> Among them, novel sesqui- and diterpenoid isocyanides, isothiocyanates and formamides are characteristic second metabolites of sea sponges of the Class Demospongia<sup>3)</sup>. Recently it has been shown that both formamides and isocyanates are biosynthesized from isocyanides<sup>4)</sup>. In the course of our program on physiologically active substances of Okinawan sea sponges<sup>5)</sup>, we isolated a novel bisabolene-type sesquiterpenoid with a conjugated diene unit, named theonellin, and its corresponding isothiocyanate and formamide from the sea sponge *Theonella* cf. *swinhoei* of the Class Demospongia, collected at Kerama Rettō, Okinawa, using SCUBA (-10 ~ -20 m). In this paper, we wish to report the structures of the sesquiterpenoids.

Methanol extraction of the fresh sea sponge, followed by ethyl acetate-water partition of the residue, furnished 0.6% of an oil which was chromatographed on a silica gel column using first chloroform-methanol (95:5) and then chloroform-methanol (9:1) as eluant by monitoring UV absorption. The less polar portion was



1



2: X=NCS

3: X=NHCHO

4: X=NHCH<sub>3</sub>

5: X= $\overset{+}{N}(CH_3)_3 \cdot OH^-$

chromatographed on a column of silica gel (ethyl acetate-n-hexane, 3:97), followed by preparative tlc (ethyl acetate-n-hexane, 1:9) to give a sesquiterpenoid, theonellin 1 (oil, 0.0093%) and its corresponding isothiocyanate 2 (oil, 0.0034%). The porlar portion gave a formamide 3 (oil, 0.018%) after successive chromatographies on a silica gel column (ethyl acetate-n-hexane, 1:1), an ODS column and a Sephadex LH-20 column (chloroform-methanol, 1:1)

Theonellin 1,  $[\alpha]_D^{25} +23^\circ$  (c 0.26, n-hexane), showed an electron impact molecular ion at  $m/z$  204 (16%,  $C_{15}H_{24}$ ) and a UV absorption maximum at 238 nm ( $\epsilon$  19000), indicating the presence of a trisubstituted conjugated diene chromophore. The  $^1H$  NMR spectrum contained signals for the conjugated diene structure  $(CH_3)_2CHCH=CH-CH=C(CH_3)-$  at  $\delta$  1.02 (6H, d,  $J=6.6$  Hz, 12-H and 15-H), 1.72 (3H, d,  $J=1$  Hz, 14-H), 2.34 (1H, octet,  $J=6.6$  Hz, 11-H), 5.54 (1H, dd,  $J=6.6, 15$  Hz, 10-H), 5.80 (1H, brd,  $J=11$  Hz, 8-H) and 6.24 (1H, ddq,  $J=11, 15, 1$  Hz, 9-H). In the  $^{13}C$  NMR spectrum ( $CDCl_3$ ) the signals for the diene part were observed at  $\delta$  14.7 (q, C-14), 22.7 (q, C-12 and C-15), 31.4 (d, C-11), 123.4 (d, C-9 or C-8), 123.9 (d, C-8 or C-9), 139.8 (d, C-10) and 140.5 (s, C-7). Configurations of the double bonds at 7 and 9 positions were determined to be E on the basis of a coupling constant between 9-H and 10-H and an ir absorption at  $970\text{ cm}^{-1}$ , and a high field resonance of C-14<sup>6)</sup>, respectively. This structure is also found in the isothiocyanate 2 and the formamide 3 as well as theonellin 1. The remained partial structure for 1 is composed of  $C_7H_{11}$  whose  $^{13}C$  NMR absorptions were observed at  $\delta$  23.5 (q, C-13), 28.0 (t, C-5), 30.7 (t, C-4 or C-1), 30.8 (t, C-1 or C-4), 43.2 (d, C-6), 120.9 (d, C-2) and 133.6 (s, C-3). Comparison of the  $^{13}C$  NMR signals with those of limonene indicated a 4-methyl-3-cyclohexen-1-yl structure. The  $^1H$  NMR spectrum contained an olefinic methyl signal at  $\delta$  1.64 (3H, brs, 13-H) and an olefinic proton signal at  $\delta$  5.38 (1H, m, 2-H). These results suggest that theonellin 1 has a bisabolene skeleton with a conjugated double bond as illustrated. The configuration at 6 position was determined to be R on the basis of the sign of its  $[\alpha]_D$ .<sup>7)</sup>

Theonellin isothiocyanate 2<sup>8)</sup> showed an electron impact molecular ion at  $m/z$  263 (94%,  $C_{16}H_{25}NS$ ). The ir spectrum ( $\nu_{\text{max}}^{CHCl_3}$   $2100\text{ cm}^{-1}$ ) and the  $^{13}C$  NMR spectrum ( $\delta$  138.7) indicated the presence of an isothiocyanate function. The  $^{13}C$  NMR spectrum ( $CDCl_3$ ) contained signals for a cyclic part at  $\delta$  27.1 (2xC, t, C-1 and C-5), 38.6 (2xC, t, C-2 and C-4), 45.0 (d, C-6) 60.9 (s, C-3) and 25.1 (q, C-13) without olefinic carbon signals indicating the symmetrical trisubstituted cyclohexane structure 2. The stereochemistry of 2 was determined by comparison with theonellin formamide 3.

Theonellin formamide 3<sup>9)</sup> showed an electron impact molecular ion at  $m/z$  249 (39%,  $C_{16}H_{27}NO$ ) and the ir spectrum showed absorption maxima at  $3280$  and  $1685\text{ cm}^{-1}$  assigned to an amide functionality. The  $^1H$  NMR spectrum contained signals for the formamide function at  $\delta$  5.98 (brs,  $NHCHO$ ) and 8.00 (d,  $J=1.7$  Hz,  $NHCHO$ ), and 7.11 (brd,  $J=12$  Hz,  $NHCHO$ ) and 8.28 (d,  $J=12$  Hz,  $NHCHO$ ), indicating that 3 exists as a 1:1 mixture of two rotational isomers of a formamide function. Reduction of 3 with LAH afforded a methylamine 4<sup>10)</sup>, which was treated with methyl iodide followed by  $Ag_2O$  to yield a quaternary ammonium base 5<sup>11)</sup>. When the quaternary base heated at  $160^\circ C$  gave a mixture of 1 and its exocyclic olefin. The GLC analysis of the reaction products

revealed that the exocyclic olefin was a predominant product, which isomerized to 1 under the condition, suggesting that the formamide group has an equatorial configuration<sup>3g</sup>), namely trans relationship to the diene part.

In the biogenetic point of view<sup>4</sup>), the isothiocyanate 2 and formamide 3 may be biosynthesized from its corresponding isocyanide originated from theonellin 1. But the isocyanide was not yet isolated from the sea sponge.

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8. 2: UV (MeOH)  $\lambda_{\max}$  238 nm ( $\epsilon$  30200);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.00 (6H, d, J=6.6 Hz, 12-H and 15-H), 1.41 (3H, s, 13-H), 1.71 (3H, d, J=1 Hz, 14-H), 2.34 (1H, octet, J=6.6 Hz, 11-H), 5.56 (1H, dd, J=15, 6.6 Hz, 10-H), 5.78 (1H, brd, J=11 Hz, 8-H) and 6.21 (1H, ddq, J=15, 11, 1 Hz, 9-H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  15.2 (q, C-14), 22.5 (2xC, q, C-12 and C-15), 31.4 (d, C-11), 123.4 (d, C-9 or C-8), 123.9 (d, C-8 or C-9), 138.7 (s, C-7) and 140.6 (d, C-10) for the diene part.

9. 3: UV (MeOH)  $\lambda_{\max}$  238 nm ( $\epsilon$  24000);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.02 (6H, d,  $J=6.6$  Hz, 12-H and 15-H), 1.38, 1.45 (each 3/2 H, s, 13-H), 1.73 (3H, brs, 14-H), 2.35 (1H, octet, 11-H), 5.55 (1H, dd,  $J=15$ , 6.6 Hz), 5.78 (1H, brd,  $J=11$  Hz), 5.98 (1/2 H, brs, exchangeable, NH), 6.22 (1H, dd,  $J=15$ , 11 Hz), 7.11 (1/2 H, brd, exchangeable, NH), 8.00 (1/2 H, d,  $J=1.7$  Hz, CHO) and 8.28 (1/2 H, d,  $J=12$  Hz, CHO);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ )  $\delta$  14.9 (q, C-14), 22.1, 24.3 (each 1/2 C, q, C-13), 22.4 (2xC, q, C-12 and C-15), 27.2 (2xC, t, C-1 and C-5), 31.2 (d, C-11), 36.7, 39.0 (each 1/2C, t, C-2 and C-4), 46.0, 46.2 (each 1/2 C, s, C-3), 123.2 (d, C-9 or C-8), 123.4 (d, C-8 or C-9), 139.1, 139.6 (each 1/2C, s, C-7), 139.8, 140.1 (each 1/2C, d, C-10), 160.4 and 162.8 (each 1/2C, d, CHO).
10. 4: EI-MS  $m/z$  235 ( $\text{M}^+$ , 34%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  1.00 (6H, d,  $J=6.6$  Hz, 12-H and 15-H), 1.07 (3H, s, 13-H), 1.72 (3H, brs, 14-H), 2.34 (3H, s,  $\text{NCH}_3$ ), 2.34 (1H, m, 11-H), 5.56 (1H, dd,  $J=15$ , 6.6 Hz, 10-H), 5.81 (1H, brd,  $J=11$  Hz, 8-H) and 6.24 (1H, dd,  $J=15$ , 11 Hz, 9-H).
11. 5: EI-MS  $m/z$  249 ( $\text{M}^+-\text{H}_2\text{O}$ , 13%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  0.94 (6H, d,  $J=6.6$  Hz, 12-H and 15-H), 1.18 (3H, s, 13-H), 1.66 (3H, brs, 14-H), 2.28 (1H, octet,  $J=6.6$  Hz, 11-H), 3.30 (9H, s,  $-\text{N}^+(\text{CH}_3)_3$ ), 5.60 (1H, dd,  $J=15$ , 6.6 Hz, 10-H), 5.70 (1H, d,  $J=11$  Hz, 8-H) and 6.13 (1H, dd,  $J=15$ , 11 Hz, 9-H).

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