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Dehydroisocyanotheonellin from a sponge Halichondria sp.

Dehydroisocyanotheonellin from a sponge *Halichondria* sp.

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Abstract

In our investigation on the secondary metabolites of Okinawan coral reef invertebrates, a series of bisabolene-class sesquiterpenoids were isolated from a sponge *Halichondria* sp. collected at Kerama Islands. After spectroscopic analyses of the isolates, new theonellin analogs **5-7** were elucidated in addition to the identification of known theonellins **1-4** and **8-9**. The experimental evidence is provided in this note.

Introduction

Marine sponges and other invertebrates living in coral reefs have been recognized as rich sources of new secondary metabolites having unique structures, often with potent biological activities.[1] On the occasion of 19th International Symposium on the Chemistry of Natural Products held at Karachi, Pakistan in 1994, one of us (TH) gave a talk on a series of new sponge metabolites: hennoxazoles,[2] miyakolide,[3] echinoclathrines,[4] mytiloxanthins[5] and others that had been isolated in our laboratory. After the symposium, a brief summary was published in a proceeding without detailed results or discussion of experimental evidence.[6] Of the new compounds in the proceeding, most of them were published,[2-5] except for hennoxazoles and theonellin-class molecules, the latter of which are the subjects of this note.

Theonellins were originally reported as bisabolene-class sesquiterpenoids from an Okinawan sponge *Theonella* cf. *swinhoei*.[7]

Following that work, similar molecules have been reported.[8-10] Among the new theonellin analogs presented by TH as of 1994, two theonellin dimers **8-9** were recently reported,[11] and **5-7** still remain to be reported as natural products. We would like to describe chemical structures and spectroscopic evidence of new sesquiterpenoids **5-7** in addition to identification of known compounds **1-4** and **8-9** isolated from the same sources. The contents are based on the undergraduate thesis of the first author (TK), prepared 30 years ago.

Results and Discussion

From the title sponge extract, we obtained a series of molecules **1-9** and a steroid **10** after chromatographic separation (Figure 1).

Compound **1** was found to contain 15 carbon signals and estimated to have a molecular formula $C_{15}H_{24}$. It was shown to be identical with theonellin after comparing the NMR data with those published.[7]

Compound **2** was shown to contain an isothiocyanate (2150 cm^{-1}, δ 135.0) with a diene moiety. By comparison with published data,[7] **2** was identified as 3-isothiocyanotheonellin.
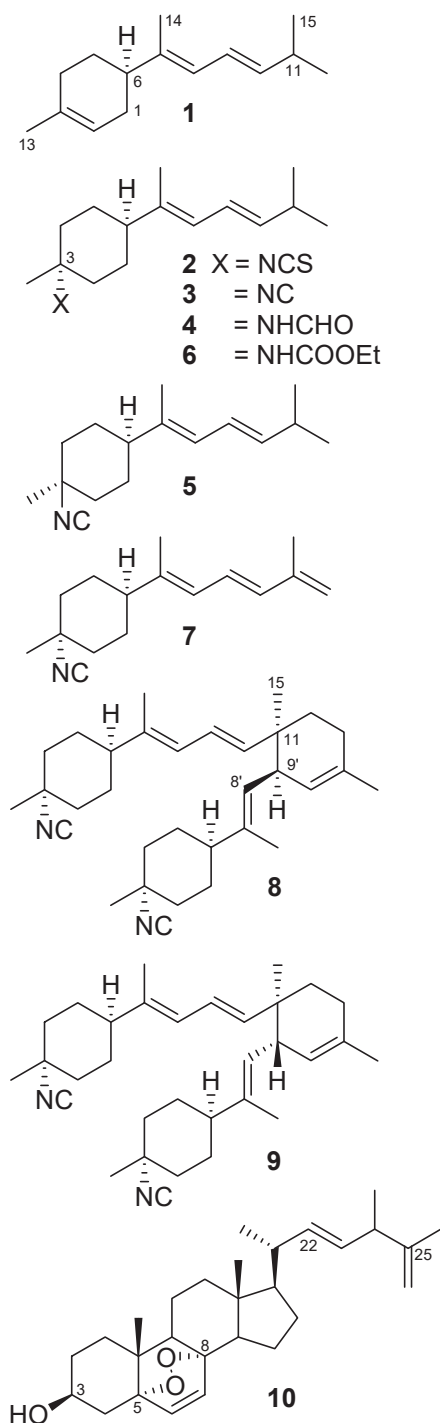

Figure 1. Structures of compounds **1-10**

Compound **3** exhibited a similar spectrum to **2**. A sharp IR absorption at 2150 cm^{-1}, unlike the broad one in **2**, a weak signal at δ 152.2, and a molecular ion at *m/z* 231, indicated that **3** has an isocyano group instead of an isothiocyanate in **2**. Therefore, the molecule was identified as 3-isocyanotheonellin after comparison.[9]

Compound **4** showed a molecular ion at *m/z* 249, 18 mass units larger than **3**, and IR absorptions at 3280 and 1685 cm^{-1} suggesting the presence of an amide group in **3** instead of an isonitrile. Although the signal resolution of ^{1}H NMR data was not high, it was identified as theonellin formamide.[7]

Compound **5** gave a molecular ion at *m/z* 231 as well as 3-isocyanotheonellin (**3**). The IR spectrum showed a sharp absorption at 2150 cm^{-1} indicating the presence of an isocyano group. ^{1}H NMR also exhibited the presence of a diene at δ 5.60 dd, 5.81 d and 6.21 ddd in addition to δ 2.34 dqq suggesting the same side chain as previous members. After assigning ^{13}C NMR chemical shifts, chemical shift differences (Δδ) between **5** and **3** were shown as in Figure 2. As larger chemical shift differences were observed around C-3, it is likely that compound **5** is epimeric at C-3.
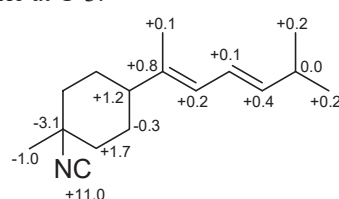

Figure 2. Δδ values for **5** and **3**

In the ^{1}H NMR spectrum of compound **6**, an ethoxy group (δ 4.06 q, 1.23 t) was observed in addition to common structural features of theonellin members: diene protons at δ 5.57 dd, 5.81 d and 6.22 ddd, two methyl doublets at δ 1.01,

and a vinyl methyl at δ 1.73 s. Two prominent IR absorptions at 1720 and 3450 cm^{-1} indicate the presence of an amide as in **4**. After comparing the ^{13}C NMR data of **6** with **4** (Figure 3), the structural difference could be placed at amide portion. By observing the molecular ion at *m/z* 293, 44 mass units larger than **4**, the structure of **6** is elucidated to have an ethoxyurethane moiety instead of a formamide in **4**.
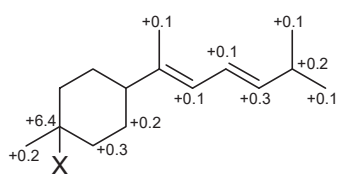
+0.1   +0.1
+0.1   +0.2
+0.1   +0.3   +0.1
+6.4   +0.2
+0.2   +0.3
X

Figure 3. Δδ values for **6** and **4**.

Compound **7** showed the presence of an isonitrile group by a sharp IR absorption at 2140 cm^{-1} and a ^{13}C NMR signal at δ 152.3, as with **3** and **5**. A molecular ion at *m/z* 229, two mass units smaller than that of **3**, and additional olefinic signals at δ 5.02 (2H, brs) and at δ 116.0 t indicated that **7** contains an exomethylene moiety. The wavelength of UV absorption maximum was 273 nm, much longer than that (239 nm) of **3**, confirming the extended double bond conjugation in **7** as we had in a similar case with a triene system of a cembrane-class diterpenoid.[12] As two vinyl methyl groups appeared at δ 1.87 and 1.99, the additional double bond can be placed at C-11. Therefore, the structure of **7** was elucidated as 11-dehydroisocyanotheonellin.

Compounds **8** and **9** showed similar spectra to each other with many more signals than those of compounds **1**-**7**. As both compounds exhibited molecular ions at *m/z* 458 corresponding to twice the molecular weight of **7**, they were suggested to be dimeric.

Compound **8** contained two isocyano groups (2140 cm^{-1}, δ 152.2 (overlapped)), a diene (δ 5.70 d, 5.79 dd, 6.14 dd), and two separate double bonds (δ 4.91 d, 5.05 d). The latter protons can be connected by analyzing COSY data from Me-14′ (δ 1.67) to H-8′ (δ 5.05), H-9′ (δ 2.75), H-10′ (δ 4.91), and finally to Me-15′ (δ 1.58). Similarly, compound **9** was found to contain two isonitrile groups (2130 cm^{-1}, δ 152.2, 152.2), a diene (δ 5.62 d, 5.78 brd, 6.17 dd), and two separate vinyl protons (δ 4.97 brd, 5.06 dd). The latter two signals were connected as well, as in **8**. Therefore, compounds **8** and **9** were assumed to be diastereomeric. As the triene **7** was isolated in a relatively good yield with unstable nature, Diels-Alder reaction might have occurred in the sponge or during extraction and isolation processes to form a cyclohexene moiety as in **8** and **9**, with different configuration. After assigning each signal, an NOE was observed between Me-15 and H-9′ in **8**, while in **9** an NOE was seen between Me-15 and H-10′ but not between Me-15 and H-9′. In conclusion, compound **8** and **9** take *cis* and *trans* configuration, respectively. Compound **8** was formed from **7** with *endo* preference, while **9** was through *exo* preference. Since they did not exhibit optical rotation, both compounds were racemates formed under simple chemical processes, and are likely to be artifacts. A search found that both **8** and **9** had recently been isolated from a Fijian sponge, with compound **7** found to be a key intermediate.[11]

Compound **10** was obtained as a crystalline molecule. ^{1}H NMR spectrum contained a characteristic signal for 3β-hydroxy group at δ 3.94 suggesting conventional steroidal nature for **10**. Additional characteristic signals were observed

for down-field-shifted olefinic protons at δ 6.22 and 6.48 which coupled mutually, a *trans*-olefin on the side chain at δ 5.22-5.23, exomethylene protons at δ 4.68, an allylic proton at δ 2.69, three singlet methyls at δ 0.80, 0.86 and 1.65 and two doublet methyls at δ 0.98 and 1.06. Compound **10** gave a molecular ion at *m/z* 426 with the help of ^{13}C NMR data, suggesting that the molecular formula is $C_{28}H_{42}O_3$. The characteristic olefinic protons at δ 6.22 and 6.48 were reminiscent of those observed for endoperoxide-containing B ring as in ergosterol peroxide.[13] The functionality can also explain the two oxygen atoms. COSY analysis gave partial structures for the side chain, i.e., H-22/23 (δ 5.22) to H-24 (δ 2.69), then to H-28 (δ 1.06). The exomethylene protons correlated with a vinyl methyl at δ 1.65. Thus, the whole structure was elucidated as 5α,8α-epidioxy-22*E*-ergosta-6,22,25-triene-3β-ol.

Experimental

General experimental conditions. NMR data were obtained on a Jeol EX270 or PMX-60 instrument. Multiplicities of ^{13}C NMR signals were as follows: s for C, d for CH, t for CH$_2$, and q for CH$_3$. EIMS spectra were obtained on a Hitachi M-2500 instrument. UV and IR spectra were obtained on a Jasco 610 spectrophotometer or on a Hitachi 260-10 spectrophotometer. Solvents used were reagent grade without distillation.

Extraction and Isolation. A specimen (0.7 kg, wet) of a sponge *Halichondria* sp. was collected at Kerama Islands, April 1987, and kept frozen until use. After extraction with acetone (1.5 L) three times, its chloroform soluble portion (6.81 g) was taken. Most of the extract (4.02 g) was applied to

vacuum flash chromatography on silica gel with stepwise elution using hexane, hexane-CH$_2$Cl$_2$ (1-1), CH$_2$Cl$_2$, and EtOAc. Each fraction was further separated with a combination of chromatography (silica gel column and reversed phase HPLC) affording compounds **1** (49.0 mg), **2** (43.6 mg), **3** (162.8 mg), **4** (95.8 mg), **5** (2.9 mg), **6** (4.7 mg), **7** (47.4 mg), **8** (14.4 mg), **9** (23.7 mg), and 5α,8α-epidioxy-22*E*-ergosta-6,22,25-triene-3β-ol (**10**, 47.2 mg).

Compound 1. Colorless oil; IR (CCl$_4$) 2950, 2920, 2830, 1430, 1380, 960 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.02 (6H, d, *J* = 6.6 Hz, H-12,15), 1.65 (3H, brs, H-13), 1.74 (3H, s, H-14), 2.35 (1H, m, H-11), 5.40 (1H, m, H-2), 5.58 (1H, dd, *J* = 6.9, 15 Hz, H-10), 5.83 (1H, d, *J* = 11 Hz, H-8), 6.25 (1H, ddd, *J* = 11, 15, 1 Hz, H-9); ^{13}C NMR (CDCl$_3$) δ 14.7 q, 22.6 q, 22.6 q, 23.5 q, 27.8 t, 30.6 t, 30.7 t, 31.4 d, 43.1 d, 120.8 d, 123.3 d, 123.7 d, 133.7 s, 139.9 d, 140.8 s.

Compound 2. Colorless oil; IR (CCl$_4$) 2930, 2860, 2100, 1460, 1440, 1380, 1125, 960 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.00 (6H, d, *J* = 6.8 Hz, H-12,15), 1.41 (3H, s, H-13), 1.70 (3H, brs, H-14), 2.35 (1H, m, H-11), 5.58 (1H, dd, *J* = 15, 7 Hz, H-10), 5.80 (1H, d, *J* = 10.8 Hz, H-8), 6.20 (1H, dd, *J* = 15, 10.8 Hz, H-9); ^{13}C NMR (CDCl$_3$) δ 15.1 q, 22.5 q, 22.5 q, 25.0 q, 27.0 t, 31.4 s, 38.5 t, 45.0 d, 60.9 s, 123.4 d, 123.8 d, 138.7 s, 140.7 d.

Compound 3. Colorless oil, [α]$_D$ 0 (*c* 0.82, CCl$_4$); IR (CCl$_4$) 2940, 2860, 2130, 1460, 1440, 1125, 960 cm^{-1}; UV (MeOH) 238 nm; ^{1}H NMR (CDCl$_3$) δ 0.99 (6H, d, *J* = 6.9 Hz, H-12,15), 1.42 (3H, brs, H-13), 1.70 (3H, d, *J* = 0.6 Hz, H-14), 2.35 (1H, m, H-11), 5.58 (1H, dd, *J* = 15, 6.6 Hz, H-10), 5.79 (1H, d, *J* = 10.6 Hz, H-8), 6.20 (1H, ddd, *J* = 15.2, 10.6, 1.3 Hz, H-9); ^{13}C NMR (CDCl$_3$) δ 15.1 q,

22.4 q, 22.4 q, 25.0 q, 26.4 t, 31.3 s, 38.2 t, 44.7 d, 56.6 s, 123.3 d, 123.8 d, 138.5 s, 140.6 d. EIMS *m/z* 231 (M⁺), 204, 93.

Compound 4. Colorless crystals; IR (CCl₄) 3430, 3220, 2950, 2930, 2850, 1680, 1490, 1460, 1320, 960 cm⁻¹; ¹H NMR (CDCl₃) δ 1.0 (6H, d), 1.3 (s), 1.4 (s), 1.7 (3H, brs), 5.6 (1H, d), 5.8 (1H, brd), 6.0 (1H, brs), 6.2 (1H, d), 8.0 (1/2H, d), 8.3 (1/2H, d); EIMS *m/z* 249 (M⁺), 204, 189, 161, 149, 105, 93.

Compound 5. Colorless oil; [α]_D 0 (*c* 0.058, CCl₄); IR (CCl₄) 2960, 2930, 2860, 2150, 1465, 1445, 1090, 960 cm⁻¹; ¹H NMR (CDCl₃) δ 1.01 (6H, d, *J* = 6.6 Hz), 1.57 (2H, s), 1.73 (3H, d, *J* = 1.3 Hz), 2.34 (1H, dqq, *J* = 6.9, 6.9, 6.9 Hz), 5.60 (1H, dd, *J* = 6.9, 15.0 Hz), 5.81 (1H, d, *J* = 10.9 Hz), 6.21 (1H, ddd, *J* = 15.0, 10.7, 1.3 Hz); ¹³C NMR (CDCl₃) δ 15.2 q, 22.6 q (2C), 24.0 q, 28.0 t, 31.5 d, 38.8 t, 45.8 d, 53.5 s, 123.4 d, 123.9 d, 139.2 s, 140.9 d, 163.2 s. EIMS *m/z* 231 (M⁺), 203, 181, 138.

Compound 6. Colorless oil; [α]_D 0 (*c* 0.094, CCl₄); IR (CCl₄) 3450, 2960, 2930, 2860, 1720, 1495, 1210, 1070, 960 cm⁻¹; ¹H NMR (CDCl₃) δ 1.01 (6H, d, *J* = 6.9 Hz), 1.23 (3H, t, *J* = 7.3 Hz), 1.60 (3H, s), 1.73 (3H, d, *J* = 0.6 Hz), 2.33 (1H, dqq, *J* = 6.6 Hz), 4.06 (2H, q, *J* = 7.0 Hz), 4.59 (1/2H, brs), 5.57 (1H, dd, *J* = 15.2, 6.9 Hz), 5.81 (1H, d, *J* = 10.9 Hz), 6.22 (1H, ddd, *J* = 15.2, 10.9, 1.2 Hz), 7.26 (1/2H, brs); ¹³C NMR (CDCl₃) δ 14.6 q, 15.0 q, 22.3 q, 22.5 q (2C), 27.4 t, 31.4 d, 37.0 t, 46.5 d, 52.4 s, 59.0 t, 123.3 d, 123.5 d, 140.2 d. EIMS *m/z* 293 (M⁺), 247, 218, 204, 189, 161, 149, 105, 93, 55.

Compound 7. Colorless oil; [α]_D 0 (*c* 0.948, CCl₄); IR (CCl₄) 2950, 2140, 1130, 960 cm⁻¹; UV (MeOH, log ε) 273 nm (4.5); ¹H NMR (CDCl₃) δ 1.54 (3H,

brs), 1.87 (3H, s), 1.99 (3H, s), 5.05 (2H, brs), 6.03 (1H, dd, *J* = 10.4, 1.3 Hz), 6.35 (1H, d, *J* = 15.5 Hz), 6.51 (1H, dd, *J* = 15.5, 10.4 Hz); ¹³C NMR (CDCl₃) δ 15.4 q, 18.5 q, 25.1 q, 26.3 t, 38.1 t, 44.9 d, 56.6 s, 116.0 t, 124.0 d, 125.2 d, 134.1 d, 141.6 s, 142.2 s, 152.3 s; EIMS *m/z* 229 (M⁺), 204, 161, 151, 121, 109, 94, 79, 55.

Compound 8. Colorless oil; [α]_D 0 (*c* 0.524, CCl₄); IR (CCl₄) 2930, 2860, 2140, 1440, 1130, 910 cm⁻¹; ¹H NMR (CDCl₃) δ 1.01 (3H, s), 1.36 (3H, s), 1.44 (3H, s), 1.58 (3H, s), 1.67 (3H, s), 1.69 (3H, s), 2.75 (1H, brd, *J* = 10.2 Hz), 4.91 (1H, brd, *J* = 10.2 Hz), 5.05 (1H, brd, *J* = 1.3 Hz), 5.70 (1H, d, *J* = 15.5 Hz), 5.79 (1H, brd, *J* = 10.6 Hz), 6.14 (1H, dd, *J* = 15.5, 10.6 Hz); ¹³C NMR (CDCl₃) δ 15.1 q, 15.2 q, 23.3 q, 25.0 q, 25.5 q, 25.9 q, 26.3 t, 26.6 t, 27.9 t, 34.0 t, 37.5 s, 37.6 t, 37.7 t, 38.1 t, 38.4 t, 44.0 d, 44.7 d, 45.0 d, 56.5 s, 56.6 s, 123.6 d, 123.7 d, 124.6 d, 126.2 d, 133.1 s, 137.1 s, 138.3 s, 139.7d, 152.2 s (2C); EIMS *m/z* 458 (M⁺), 433, 431, 418, 404, 229, 202, 134, 119, 107.

Compound 9. Colorless oil; [α]_D 0 (*c* 0.212, CCl₄); IR (CCl₄) 2930, 2860, 2130, 1440, 1130, 910 cm⁻¹; ¹H NMR (CDCl₃) δ 0.89 (3H, s), 1.43 (3H, s), 1.44 (3H, s), 1.56 (3H, d, *J* = 1.3 Hz), 1.65 (3H, brs), 1.70 (3H, d, *J* = 1.0 Hz), 2.82 (1H, brd, *J* = 10.2 Hz), 4.97 (1H, brd, *J* = 10.2 Hz), 5.06 (1H, dd, *J* = 1.7 Hz), 5.62 (1H, d, *J* = 15.4 Hz), 5.78 (1H, brd, *J* = 10.5 Hz), 6.17 (1H, dd, *J* = 10.5, 15.3 Hz); ¹³C NMR (CDCl₃) δ 15.1 q, 15.2 q, 20.8 q, 23.4 q, 25.1 q, 25.5 q, 26.5 t, 26.6 t, 27.7 t, 33.4 t, 37.7 t, 38.0 s, 38.0 t, 38.1 t, 38.3 t, 43.2 d, 44.7 d, 44.9 d, 56.7 s, 56.8 s, 122.3 d, 123.7 d, 124.4 d, 124.9 d, 132.9 s, 137.7 s, 138.5 s, 143.0 d, 152.2 s, 152.2 s. EIMS *m/z* 458 (M⁺), 447, 431, 404, 229, 202, 134, 107.

Ergosterol derivative 10. White crystals, mp 141-

142 C; $[\alpha]_D$ -8.4 (c 0.596, CCl$_4$); IR (CCl$_4$) 2950, 2140, 1130, 960 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 0.80 (3H, s), 0.86 (3H, s), 0.98 (3H, d, J = 6.6 Hz), 1.06 (3H, d, J = 6.6 Hz), 1.65 (3H, brs), 2.69 (1H, m), 3.94 (1H, m), 4.68 (2H, brs), 5.22 (1H, dd, J = 15.2, 5.2 Hz), 5.23 (1H, dd, J = 15.2, 5.9 Hz), 6.22 (1H, d, J = 8.6 Hz), 6.48 (1H, d, J = 8.6 Hz); ^{13}C NMR (CDCl$_3$) δ 13.2 q, 18.6 q, 19.2 q, 20.8 s, 21.0 q, 21.0 q, 23.8 t, 29.0 t, 30.1 t, 35.1 t, 37.3 t, 37.3 t, 39.7 t, 39.9 d, 44.0 d, 45.0 s, 51.5 d, 52.0 d, 56.5 d, 66.7 d, 79.8 s, 82.6 s, 109.3 t, 131.1 d, 132.3 d, 135.7 d, 135.9 d, 150.1 s; EIMS m/z 426 (M$^+$), 408, 390, 374, 251, 123, 81.

Acknowledgements

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