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Structures of nine oxygenated 4-methylene sterols from Hachijo marine sponge *Theonella swinhoei*

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Nine new sterols have been isolated from a marine sponge, Theonella swinhoei. *Of these, seven sterols were found to be conicasterol derivatives [(24R)-4-methylene-24-methyl-8(14)-cholesten-3β-ol] oxygenated at carbon-7 and/or carbon-15. The other two were (24R)-7β,8β-epoxy-14α-methoxy-4-methylene-24-methylcholestan-3β-ol and (24R)-8,14-seco-8,14-dioxo-4-methylene-24-methylcholestan-3β-ol.* (*Steroids* **60**:738–742, 1995)

Keywords: marine sterol; 4-methylene sterol; conicasterol derivatives; *Theonella swinhoei*; marine sponge

Introduction

Conicasterol (**1**) and theonellasterol (**2**) are biosynthetically unusual 4-methylene sterols which have been isolated from the Red Sea marine sponges *Theonella conica* and *Theonella swinhoei*.[1] The structure of **1** was deduced by comparison of its spectroscopic data with that of 4-methylenecholestan-3β-ol. The relative stereochemistry of the 24-methyl group was estimated by comparison of its ^{13}C NMR data with those of 24*R*- and 24*S*-methylcholestane derivatives. The corresponding 3-keto-4-methylene sterols, conicasterone (**3**) and theonellasterone (**4**), have been isolated along with **1** and **2** from the Okinawan marine sponge *T. swinhoei*.[2] Bistheonellasterone (**5**) has also been isolated through a Diels-Alder type cycloaddition of theonellasterone (**3**) to an isomeric $\Delta^{4(5)}$-theonellasterone.[2]

Recently, we isolated conicasterol (**1**), conicasterone (**3**), and bisconicasterone (**6**) as major constituents from the marine sponge *T. swinhoei*, which was collected from Nazumado bay on Hachijo Island.[3] Compound **6** was also produced in vitro by a Diels-Alder–type dimerization of **3**. The relative and absolute stereochemistries of **1** were unambiguously confirmed by a modified Mosher's method and X-ray crystal analysis. Through a further investigation of the Hachijo sponge, we isolated several oxygenated conicasterols from fractions of higher polarity as minor constituents. This report describes the structures of these sterols.

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Experimental

General

Melting points were uncorrected. IR spectra were taken on a JASCO IR Report-100 spectrometer. UV spectra were recorded on a Hitachi 340 spectrophotometer. ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ on a Brucker AM-500 spectrometer; chemical shifts were recorded relative to TMS, the internal standard. Specific rotations were measured on a JASCO DIP-181 polarometer. Column chromatographies were performed using Merck Silica gel 60 or Merck Aluminiumoxid 90, while flash chromatographies were performed using a Wakogel C-300 or Wako Alumina (300 mesh) with the stated solvent. Gas chromatography-mass spectroscopy (GC-MS) spectra were obtained on a Shimazu QP-2000 spectrometer, while HRMS (EI) spectra were observed on a JEOL JMS-DX-300 mass spectrometer (the ionization energy was 30 eV). Microanalyses were performed at the Analytical Center, University of Tsukuba.

Isolation

The marine sponge *T. swinhoei* (22 kg, wet weight), collected by Nazumado Bay on Hachijo Island, was homogenized. The filtrate was extracted with EtOH. The extract was concentrated under reduced pressure, and the residue was partitioned between Et_2O and H_2O. The Et_2O extract (120 g) was further partitioned between *n*-hexane and 90% aqueous MeOH. A portion (5.0 g) of the *n*-hexane extract (52.4 g) was subjected to flash chromatography on silica gel with *n*-hexane/AcOEt (4:1, v/v). The fraction eluting earlier (1.4 g) was separated by column chromatography on alumina to give bisconicasterone (**3**, 35 mg, 0.7% based on the *n*-hexane extract) and conicasterone (**2**, 130 mg, 2.6%). The fraction (0.7 g) which eluted subsequently gave conicasterol (**1**, 285 mg, 5.7%) as colorless needles upon recrystallization from MeOH.

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The more polar fraction (1.9 g) was chromatographed on silica gel by stepwise elution with increasing amount of AcOEt in *n*-hexane. The fraction (0.3 g) which eluted with 70% (v/v) AcOEt/*n*-hexane yielded **7** (100 mg, 2.0%) after precipitation from CH_2Cl_2. The residual CH_2Cl_2 soluble portion was fractionated by flash chromatography on alumina followed by preparative thin-layer chromatography (TLC) on alumina and/or silica gel to give **8** (3 mg, 0.06%), **9** (13 mg, 0.26%), **10** (19 mg, 0.38%), **11** (12.5 mg, 0.25%), **12** (1.5 mg, 0.03%), **13** (9 mg, 0.18%), **14** (4.5 mg, 0.09%), and **15** (1.5 mg, 0.03%).

7. m.p. 160–161°C (MeOH); $[\alpha]_D^{20}$ + 9.4° (c = 0.16, $CHCl_3$); EIMS m/z 428 (M^+, 83), 410 (100), 395 (22), 377 (12), 301 (9), 283 (84), and 149 (27); ^{13}C and ^1H NMR data, see Tables 1 and 2. Found: M^+, m/z 428.3651. Calculated for $C_{29}H_{48}O_2$: m/z 428.3652.

8. m.p. 160.5–161°C (MeOH); ^{13}C and ^1H NMR data, see Tables 1 and 2. Found: C 81.19, H 11.46%. Calculated for $C_{30}H_{50}O_2$: C 81.39, H 11.38%.

9. $[\alpha]_D^{20}$ + 27.0° (c = 0.23, $CHCl_3$); EIMS m/z 443 (M^+ − CH_3, 5), 426 (39), 408 (8), 393 (1), 375 (4), 299 (100), 281 (10), and 149 (26); ^{13}C and ^1H NMR data, see Tables 1 and 2. Found: M^+ − CH_4O, m/z 426.3494. Calculated for $C_{29}H_{46}O_2$: m/z 426.3495.

10. $[\alpha]_D^{20}$ + 34.1° (c = 0.13, $CHCl_3$); EIMS m/z 458 (M^+, 7), 443 (26), 440 (20), 426 (16), 408 (9), 393 (1), 375 (1), 299 (100), 281 (12), and 149 (44); ^{13}C and ^1H NMR data, see Tables 1 and 2. Found: M^+ − H_2O, m/z 440.3649. Calculated for $C_{30}H_{48}O_2$: m/z 440.3652.

11. EIMS m/z 444 (M^+, 4), 426 (100), 408 (56), 393 (37), 375 (16), 299 (85), 281 (32), and 149 (10); ^{13}C and ^1H NMR data, see Tables 1 and 2. Found: M^+ − H_2O, m/z 426.3489. Calculated for $C_{29}H_{46}O_2$: 426.3495.

12. IR (CCl_4) 1721 cm^{-1}; ^{13}C and ^1H NMR data, see Tables 1 and 2. Extra peaks not shown in Table 1: 35.12 (t), 32.00 (t), 29.77 (t), 29.74 (t), 29.69 (t), 29.58 (t), 29.44 (t), 29.36 (t), 29.21 (t), 25.05 (t), 22.77 (t), 22.74 (q?), 14.20 (q).

13. ^{13}C and ^1H NMR data, see Tables 1 and 2. Found: M^+ − H_2O, m/z 410.3542. Calculated for $C_{29}H_{46}O$: m/z 410.3546.

14. ^{13}C and ^1H NMR data, see Tables 1 and 2; EIMS m/z 426 (M^+ − CH_4O, 45), 408 (26), 393 (20), 375 (12), 299 (39), and 291 (100). Found: M^+ − CH_4O, m/z 426.3493. Calculated for $C_{29}H_{46}O_2$: m/z 426.3495.

15. IR (CCL_4) 1730 and 1710 cm^{-1}; ^{13}C and ^1H NMR data, see Tables 1 and 2; EIMS m/z 444 (M^+, 11), 429 (10), 237 (39), 224 (21), 179 (38), 149 (24), and 97 (100). Found: M^+, m/z 444.3604. Calculated for $C_{29}H_{48}O_3$: m/z 444.3601.

Methanolysis of the ester 12

To a solution of **12** (5.9 mg, 9.4 μmol) in dry MeOH (0.5 mL), 2 Eq of NaOMe/MeOH were added, and the mixture was stirred at

Table 1 ^{13}C NMR Data of **1** and **7–16** in $CDCl_3$

C	1	7	8	9	10	11	12	13	14	15	16
1	36.82	36.45	36.48	36.50	36.64	36.50	36.45	36.62	38.88	36.68	36.87
2	33.28	33.04	33.09	32.97	32.99	33.03	32.95	33.04	32.53	32.34	32.84
3	73.47	73.29	73.32	73.23	73.32	73.27	73.24	73.30	73.14	73.01	73.16
4	153.24	152.40	152.92	152.62	152.44	152.49	152.27	152.74	152.44	151.01	152.10
5	49.55	42.74	42.87	42.72	42.33	42.71	42.49	49.36	39.48	48.51	49.30
6	24.75	27.40	30.21	30.22	31.84	30.97	30.49[a]	24.90	25.29	23.75	25.22
7	29.45	79.86	74.33	66.66	65.30	66.76	66.77	31.07	56.54	41.73	27.30
8	125.76	122.55	124.40	132.27	134.54	135.84	136.97	137.87	64.61	211.19	140.62
9	49.34	44.73	43.61	45.05	43.84	45.14	44.48	49.56	42.90	46.76	50.87
10	40.08	39.73	40.11	39.56	40.16	39.61	39.95	40.43	37.05	44.39	41.87
11	20.51	20.01	19.95	19.85	19.79	19.81	19.80	20.49	21.64	18.13	20.13
12	37.44	36.94	37.03	37.34	37.37	37.48	37.17	37.25	30.59	37.41	37.16
13	42.83	43.62	43.51	42.85	42.29	43.38	43.42	42.84	48.30	52.64	42.65
14	143.02	153.65	149.46	149.09	146.83	151.55	146.49	140.37	89.36	224.96	149.79
15	25.88	25.63	25.67	80.19	77.93	70.42	73.58	84.41	30.83	38.03	208.09
16	27.13	26.88	27.01	32.97	34.91	39.33	36.79	33.52	22.55	26.13	42.52
17	56.95	56.72	57.15	53.43	54.06	53.30	53.43	53.50	51.70	62.66	50.82
18	18.30	18.03	17.74	19.77	18.63	19.72	19.43	19.56	16.70	18.36	18.81
19	13.28	12.45	12.59	12.65	12.51	12.65	12.57	13.45	16.09	13.12	13.52
20	34.63	34.58	34.66	33.87	34.70	33.88	33.78	34.01	35.96	34.48	34.71
21	19.16	19.12	19.19	19.10	19.16	19.09	19.03	19.11	18.90	18.59	19.32
22	33.62	33.54	33.62	33.61	33.48	33.71	33.50	33.66	33.97	32.45	33.52
23	30.26	30.26	30.51	30.32	30.20	30.41	30.27[a]	30.60	29.76	30.77	30.00
24	39.02	39.00	39.01	38.99	39.01	39.02	38.90	39.00	38.93	39.01	38.91
25	32.47	32.47	32.47	32.50	32.46	32.44	32.44	32.46	32.48	32.47	32.44
26	20.29	20.28	20.29	20.26	20.26	20.29	20.23	20.28	20.26	20.29	20.24
27	18.28	18.31	18.33	18.38	18.29	18.33	18.32	18.35	18.37	18.29	18.27
28	15.46	15.46	15.46	15.52	15.47	15.48	15.51	15.52	15.47	15.51	15.44
29	102.86	103.11	102.40	102.88	103.11	102.97	103.03	103.12	103.33	104.33	103.60
OMe			54.46	56.25	55.81				51.91		
COO							173.44[b]				

[a]These are interchangeable.
[b]For peaks on the acid part, see Experimental.

Table 2 Selected ^{1}H NMR data of **1, 7–15,** and **18** in $CDCl_3$[a]

H	1	7	8	9	10	11	12	13	14	15	18
3	4.009 dd (10.9, 5.3)	4.013 dd (10.8, 6.4)	4.025 dd (10.5, 6.4)	4.027 dd (10.6, 5.4)	4.029 dd (10.8, 6.4)	4.041 m	4.04 m	4.012 dd (10.7, 5.6)	3.978 m	4.057 dd (11.5, 5.7)	4.03 m
5	1.79	2.155 dd (12.8, 2.6)	2.259 dd (11.1, 2.5)	2.295 dd (12.7, 2.0)	2.339 dd (13.0, 2.0)	2.319 dd (11.1, 2.5)	2.35 dd (12.1, 2.0)	1.855 dd (12.0, 1.6)	2.326 dd (11.8, 3.0)	2.269 dd (11.6, 2.6)	
7		4.835 t (3.0)	4.111 t (2.9)	4.403 dd (3.6, 2.6)	4.837 t (3.1)	4.689 t (3.2)	4.32		3.159 d (3.0)		
9	1.79	2.113	2.215	2.359	2.319	2.364	2.41	1.926	2.01	1.96	
15				4.309	4.281	4.902	5.29	5.019			4.65
17	1.12	1.240	1.176	1.418	1.090	1.582		1.433	1.79	2.10	
18	0.826	0.854	0.844	0.797	0.958	0.812	0.833	0.829	0.782	0.850	1.010
19	0.578	0.574	0.570	0.576	0.588	0.600	0.590	0.628	0.716	0.514	0.611
21	0.917	0.926	0.936	0.930	0.916	0.948	0.940	0.924	0.860	1.069	0.915
26	0.841	0.842	0.846	0.846	0.840	0.847	0.850	0.847	0.840	0.856	0.841
27	0.791	0.792	0.796	0.798	0.790	0.797	0.788	0.799	0.793	0.808	0.791
28	0.767	0.766	0.772	0.783	0.772	0.774	0.755	0.786	0.764	0.794	0.767
29	5.062	5.097	5.053	5.089	5.081	5.097	5.09	5.080	5.103	5.149	5.10
	4.619	4.617	4.575	4.607	4.600	4.631	4.60	4.633	4.672	4.665	4.64
OMe			3.169	3.321	3.253				3.367		

[a]Coupling constants are shown in parentheses.

room temperature for 23 h. The reaction mixture was evaporated under reduced pressure. The residue was partitioned into Et_2O/H_2O and extracted twice with ether. The ether extracts were washed with brine and dried over $MgSO_4$. After the solvent was evaporated, the residue (6.2 mg) was subjected to flash chromatography on silica gel (*n*-hexane/AcOEt, 7:3, v/v). This resulted in a mixture of fatty acid methyl esters (1.8 mg) and the starting compound **12** (0.9 mg). Elution with *n*-hexane/AcOEt (7:3, v/v) gave **11** (1.6 mg), which was identified by ^{1}H NMR spectrum. The methyl ester fractions were analyzed by GC – MS with a column temperature of 150–200°C and a progress rate of 5°C min^{-1}.

15-Oxoconicasterol (16)

Compounds **9, 10,** and **13** were fairly unstable, and when their CH_2Cl_2 solutions were left in the refrigerator for half a year, they gradually changed into 15-oxoconicasterol **(16);** 4.6 mg of **16** from 37.0 mg of **10** (preparative TLC on silica gel, *n*-hexane/AcOEt, 3:2, v/v, 3 times); 1.7 mg of **16** from 9.0 mg of **13** (silica gel column, *n*-hexane/AcOEt, 7:3, v/v). Formation of **16** from **9** was recognized by a TLC spot.

16. IR ($CHCl_3$) 1693 cm^{-1}; UV (MeOH) 255 nm; ^{1}H NMR 5.095 and 4.663 (29 H_2), 4.197 (dd, J = 10.0 and 2.6 Hz, 7 Heq), 4.033 (dd, J = 11.0 and 5.5 Hz, 3 H), 2.003 (9 H), 1.963 (5 H), 1.558 (7 Hax), 1.474 (17 H), 0.987 (d, 21 Me), 0.958 (s, 18 Me), 0.840 (d, 26 Me), 0.790 (d, 27 Me), 0.760 (d, 28 Me), and 0.609 (s, 19 Me); ^{13}C NMR data, see Table 1; EIMS m/z 426 (M^+, 28), 408 (12), 393 (17), 299 (4), 281 (11), 149 (40), and 57 (100). Found: M^+, m/z 426.3504. Calculated for $C_{29}H_{46}O_2$:426.3495.

Reduction of 15-oxoconicasterol (16)

15-Oxoconicasterol (**16,** 2.9 mg) was treated with excess $NaBH_4$ in EtOH in the presence of 1.1 Eq $CeCl_3 \cdot 7H_2O$, and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into water and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with brine and dried over $MgSO_4$. After evaporating the solvent, the residue was flash chromatographed on silica gel (*n*-hexane/AcOEt, 4:1, v/v) to give 15β-hydroxyconicasterol (**18,** 0.2 mg) and its dehydrated compound (**17,** 0.9 mg).

17. UV (MeOH) 239 nm; ^{1}H NMR 5.385 (15 H), 5.091 and 4.695 (29 H_2), 4.023 (m, 3 H), 0.927 (d, J = 6.3 Hz, 21 Me), 0.867 (s, 18 Me), 0.847 (d, J = 6.8 Hz, 26 Me), 0.817 (s, 19 Me), 0.799 (d, J = 6.8 Hz, 27 Me), and 0.776 (d, J = 6.5 Hz, 28 Me). Found: M^+, m/z 410.3538. Calcd for $C_{29}H_{46}O$: 410.3546. 15β-hydroxyconicasterol **(18):** ^{1}H NMR, see Table 2.

Results and discussion

The ethanol extract of the sponge was partitioned in ether-water, and the resulting ether extract was partitioned again in hexane-90% methanol. The hexane extract was chromatographed repeatedly on silica gel and/or alumina to give nine new 4-methylene sterols, **7–15.** Of these, compounds **7–13** were each found to have a 4-methylene group, a 3β-hydroxyl group, and a double bond between C-8–C-14. The additional functional groups were either a hydroxyl or a methoxyl.

Before elucidation of the new sterols, we reinvestigated the assignment of signals in ^{1}H and ^{13}C NMR spectra of conicasterol (**1**) for precision. Eight partial structures were found from the H-H, C-H COSY, and HOHAHA spectra [see Figure 2(A), R = H]. These partial structures were connected by long-range C-H couplings (COLOC spectrum, curved arrows shown). The assignments are shown in Tables 1 and 2. The reported[1] ^{13}C chemical shifts of C-1, C-6, C-15, C-16, and C-20 should be revised as shown in Table 1.

Compound **7** was determined to be $C_{29}H_{48}O_2$ from HRMS analysis. The extra oxygen was identified as a hydroxyl group from δ values at 79.86 (d) in ^{13}C and at 4.835 (t, J = 3 Hz) in ^{1}H NMR spectra. The plane structure of **7** was elucidated by 2D-NMR analyses, H-H and C-H COSY, and COLOC [see Figure 2(A), R = OH] and by comparison of its NMR spectra with those of conicasterol **(1).** Compound **7** was thus found to be 7-hydroxyconicasterol.

Similar procedures were performed for compounds **8–11.** The location of hydroxyl and/or methoxyl group(s) was determined as shown by 2D NMR analyses. The as-

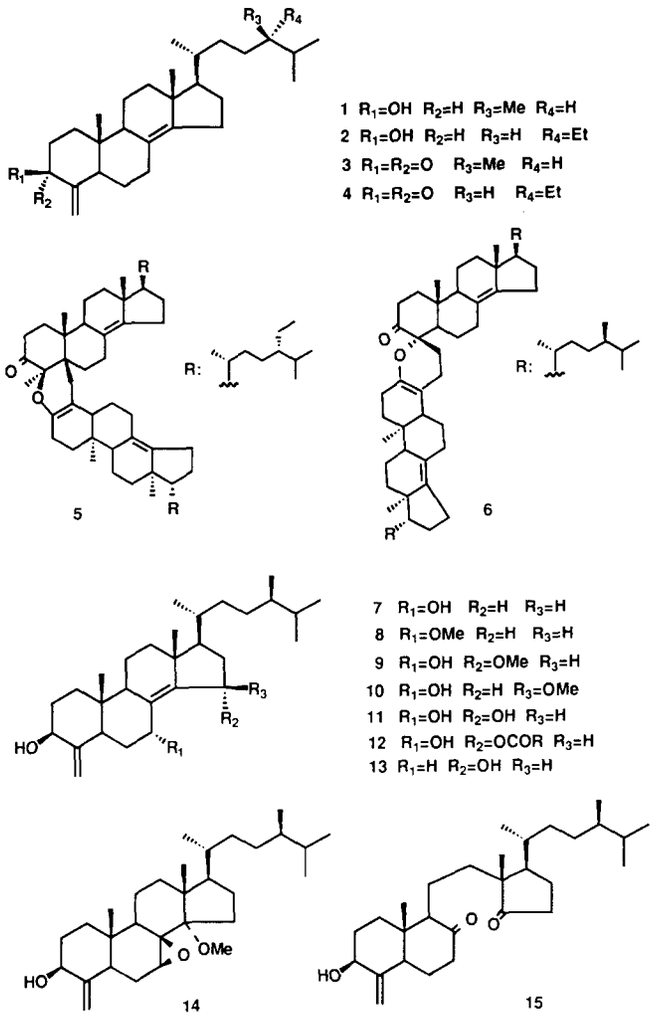


Figure 1 Structures of the known 4-methylene sterols **1–6** and new 4-methylene sterols **7–15.**

signments of ^{13}C and selected ^{1}H NMR signals are summarized in Tables 1 and 2.

Compound **12** showed the presence of an ester moiety (ν 1721 cm^{-1} in IR and δ 173.44 in ^{13}C NMR). The ^{13}C NMR spectrum of **12** was very similar to that of **11** except for carbons C-14, C-15, and C-16. Several extra peaks (at least 7) were recognized around 29 ppm along with peaks at 25.04 (t) and 35.12 (t), which were assigned to $-CH_2CH_2COO-$. Moreover, the somewhat broad signals at 14.20 (q), 22.77 (t), and 32.00 (t) were assigned to $CH_3CH_2CH_2-$ of a long-chain fatty acid. When **12** was subjected to methanolysis, **11** was obtained. Thus, the structure of **12** was identified as the monoacyl compounds of **11,** and the position of its acyl group was localized to C-15.

The methyl ester fractions from the methanolysis of **12** were found to contain at least three components via GC analysis; the retention times of these components were 14.07, 14.26, and 15.25 min in relative amounts 1.0:0.5:0.4. The retention time of the major component was just between those of methyl myristate (12.98 min) and methyl palmitate (18.26 min). GC-MS analysis of the components identified the major constituent as methyl pentadecanoate

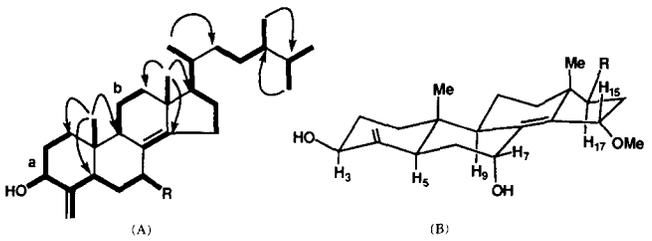
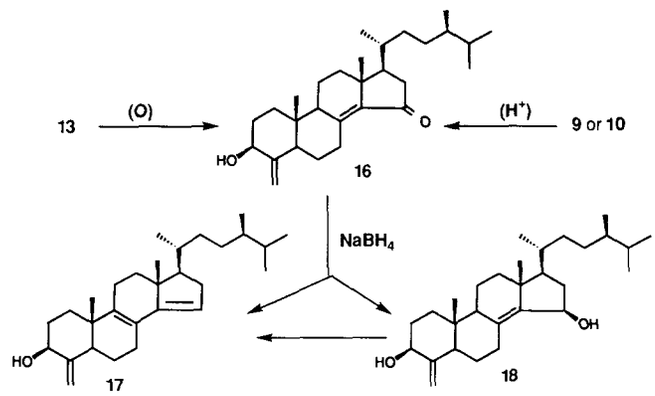


Figure 2 (A) 2D-NMR analysis of **1** (R=H) and **7** (R=OH). Curved arrows show selected long-range C-H couplings. (B) Stereochemistry of **9.**

[m/z 256 (M^+, 5%), 87 (67%), 74 (base) along with 11 continuous $-CH_2$ peaks starting from m/z 241]. This unusual C_{15} fatty acid has previously been isolated from algae.[4] The structures of the minor constituents remain uncertain, but they have olefinic double bond(s) as revealed by the presence of a weak broad peak at δ 5.33 (relative intensity was 0.37 H) in the ^{1}H NMR spectrum of **12.**

Compound **13** was determined to be $C_{29}H_{48}O_2$ from HRMS analysis. The presence of an extra hydroxyl group was clarified from δ 5.019 in ^{1}H NMR and δ 84.41 in ^{13}C NMR spectra. The 2D-NMR analyses identified compound **13** as 15-hydroxyconicasterol.

The stereochemistry of C-7 in compounds **7–12** could be easily deduced from the vicinal coupling constants of C7-H, which show the hydroxyl (or methoxyl) group in an axial position. This was also confirmed by the low-field shifts of C5-H and C9-H due to the 1,3-diaxial relationships of these protons to C7-OH (or OMe; see Figure 2B). On the other hand, the stereochemistry at C15-OH (or OMe) in compounds **9–13** was slightly ambiguous because no information could be obtained from the coupling pattern around the five-membered ring. However, a distinct homoallylic coupling between C9-H and C15-H was observed in **9** and **11–13** but not in **10.** This shows that the stereochemistry of **10** is different from those of the others. The chemical shift of C17-H in **10** is similar to that of conicasterol **(1)** but its C13-Me shifts to a lower field. The reverse situation was observed in **9** and **11–13.** These facts are in accordance with the finding that the methoxyl group in **10** is in a β-position, while the hydroxyl (or methoxyl) group in the other sterols is in an α-position.



Scheme 1 Formation of **16** from **9, 10,** or **13** and the $NaBH_4$ reduction of **16.**

Both **9** and **10** were gradually changed to 15-oxo-conicasterol **(16)** when CH_2Cl_2 solutions of these compounds were left standing; **16** was probably formed by elimination of the 7α-hydroxyl group to give a 15-methoxy-$\Delta^{7,14}$-diene, which was then hydrolyzed at its enol ether moiety. Compound **16** was also derived from **13** by air-oxidation. The usual chemical shift [δ 4.197 dd (J = 10.0 and 2.6 Hz)] of the equatorial proton of C-7 in **16** is due to the deshielding effect of the carbonyl group. Sodium borohydride reduction of **16** gave the diene **17** and an unstable alcohol **18.** The facile conversion of **18** into the diene **17** was observed when **18** was allowed to stand. Although complete analysis of the NMR spectra of **18** could not be attained due to instability of the compound, the ^{1}H chemical shift of C13-Me was observed at δ 1.010, which shows that the hydroxyl group is in a β-configuration as in **10.** The stereochemistry of the reduction is in accordance with that of 15-ketosteroids.[5] The stereochemistry around the side-chain was determined to be the same as that of conicasterol **(1)** by comparison of ^{13}C chemical shifts. The chemical shift difference (Δδ) between the C-26 and C-27 methyl carbons was in accordance with the reported value; Δδ is 2 ppm when the 24-methyl group is in an *R*-position, while it is 3 ppm when the methyl group has an *S*-configuration.[6] (We assigned the chemical shift of C-26 as lower than that of C-27 in 24*R*-methyl-side chain based on the result from (2*R**,3*R**)-1-deutero-2,3-dimethylhexane. The assignment was coincident with that from the biosynthetic results.[7])

Compound **14** has a 3β-hydroxyl and a 4-methylene group but no double bond between C-8 and C-14. One methoxyl group attaches at the quaternary carbon [δ 51.93 (q) and 89.36 (s) in ^{13}C NMR]. HRMS revealed the molecular formula as $C_{30}H_{50}O_3$. The remaining oxygen atom forms an epoxide ring as estimated from NMR spectra [δ 3.159 as doublet (J = 3.0 Hz) in ^{1}H NMR; 56.54 (d) and 64.61 (s) in ^{13}C NMR]. The 2D NMR analyses clarified **14** as a 7,8-epoxy-14-methoxysterol derivative. The stereochemistry of the epoxide ring was deduced as β from the H-H coupling patterns around C-5–C-6–C-7. The methine proton on the epoxide ring couples only with the equatorial proton on C-6 [δ 1.989 (dt, J = 11.8 and 3.0 Hz, H-6e) and 1.782 (t, J = 11.8 Hz, H-6a)]. Examination of a Dreiding model shows that this situation is possible only when the epoxide ring is in a β-position. When the epoxide ring is in an α-position, the methine proton should couple either with both protons on C-6 if the C-ring is in a chair conformation or with the axial proton on C-6 if the C-ring is in a boat conformation. Lowfield shift of C17-H in ^{1}H NMR spectrum requires the methoxyl group to be in an α-position.

The last compound, **15,** $C_{29}H_{48}O_3$, has two carbonyl groups as revealed from IR (ν 1730 and 1710 cm^{-1}) and ^{13}C NMR (δ 224.96 and 211.19) spectra. The C-8–C-14 seco structure with carbonyl groups at C-8 and C-14 was established from 2D NMR spectra and EIMS. Two MacLafferty peaks (m/z 224 and 194) from both carbonyl groups and the peak (m/z 237) from the fission between C-11 and C-12 agree with the proposed structure. Jereisterol B[8] has been described as the only C-8–C-14 seco sterol from marine organisms. Compound **15** is a second example of such an unique structure.

Mass spectra of the present sterols revealed several characteristic features. While M^+ ion appeared as a base peak in conicasterol **(1),**[1] m/z 299 (M^+ − ROH − side chain) appeared as a base peak (**9** and **10**) or strong peak **(11)** in 15-alkoxy-$\Delta^{8(14)}$-sterols. The M^+ ions were very weak or unrecognized in these compounds. The unsaturation (probably $\Delta^{15(16)}$ due to the elimination of ROH) in the D-ring will cause this feature.

It is interesting that neither theonellasterol **(2)** nor its derivatives could be found in the Hachijo sponge *T. swinhoei*. These compounds have been isolated from both the Red Sea[1] and Okinawan[2] *T. swinhoei*.

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