Marine Natural Products. XXX.[1] Two New 3-Keto-4-methylene Steroids, Theonellasterone and Conicasterone, and a Diels–Alder Type Dimeric Steroid Bistheonellasterone, from the Okinawan Marine Sponge *Theonella swinhoei*

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Two new 3-keto-4-methylene steroids, theonellasterone (3) and conicasterone (4), and a Diels–Alder type dimeric steroid, bistheonellasterone (5), were isolated together with two known 4-methylene sterols, theonellasterol (1) and conicasterol (2), from the Okinawan marine sponge *Theonella swinhoei*. The structures of these steroids have been elucidated on the basis of chemical and physicochemical evidence. Bistheonellasterone (5) is considered to be biosynthesized through a Diels–Alder cycloaddition of theonellasterone (3) and its Δ^4-isomer. Very interestingly, theonellasterone (3) and conicasterone (4) were seen under an optical microscope as crystals deposited in the tissue of fresh marine sponge.

Keywords marine sponge; *Theonella swinhoei*; theonellasterone; conicasterone; bistheonellasterone; steroid 4-methylenated; steroid dimeric; Diels–Alder cycloaddition

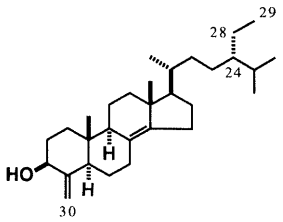
Recent chemical investigations of marine sponges have disclosed that *Theonella* species are rich sources of biologically active secondary metabolites: *e.g.* aminobisabolenes,[2] onnamide A,[3] theonellamide F,[4] theonelladines A—D,[5] and misakinolide A (=bistheonellide A).[6,7] During the course of our investigations in search of new biologically active substances from marine organisms, we have analyzed the constituents of the Okinawan marine sponge *Theonella swinhoei* and have isolated several bioactive tridecapeptide lactones, named theonellapeptolides Ia—e,[8,9] and four potent cytotoxic dimeric macrolides, named swinholides A, B, C and isoswinholide A.[10–13]

In the structure study of swinholides, we have noticed, as described in our preceding paper,[11] that the atomic array in the structure of the monomeric unit of swinholide A is very similar to that in scytophycin C, which was previously isolated by Moore and his group[14] from the cultured terrestrial blue-green alga *Scytonema pseudohofmanni*. Since then, we have been interested in the possible contribution of either symbiotic or parasitic microorganism(s) presumably existing in the marine sponge *Theonella swinhoei* to the biosynthesis of swinholide A and its congeners. First, we have investigated the tissue of this particular kind of marine sponge by means of electron microscope analysis and we have found many files of what is possibly a blue-green alga growing in the marine sponge. We have also investigated the tissue using an optical microscope and very interestingly we found deposits of many crystals together with many files of a blue-green alga in the tissue of fresh marine sponge.

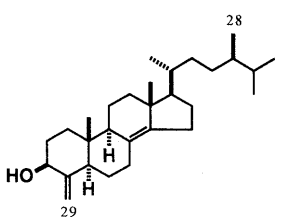
In order to characterize the chemical composition of these crystals, we re-investigated in more detail the chemical constituents of the lipid-soluble portion of the Okinawan marine sponge *Theonella swinhoei*. Eventually, we found two new 3-keto-4-methylene steroids named theonellasterone (**3**) and conicasterone (**4**), portions of which are separated out as crystals in the tissue of fresh marine sponge, and a dimeric steroid named bistheonellasterone (**5**), together with two known 4-methylene sterols, theonellasterol (**1**) and conicasterol (**2**).[15] In this paper, we present a full account of the structure elucidation of these 4-methylene steroids.

As mentioned above, we have found by microscopic observation the deposition of many crystals in the tissue of fresh marine sponge, which was collected in May at Hedo Cape, Okinawa. In order to identify the chemical constituents of these crystals, we isolated them through the following procedure. Thus, the fresh marine sponge was cut into pieces and dispersed in sea water. Then, the whole was filtered through gauze to remove tissue particles, and the filtrate was centrifuged (<1000 rpm). The crystals, which were floating in the resulting supernatant, were collected on a filter paper.

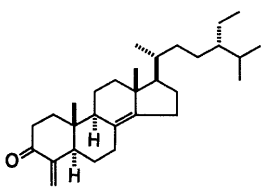
On the other hand, the lipophilic fraction of the acetone extract of the marine sponge (AcOEt ext. in Fig. 1) was treated with *n*-hexane–AcOEt (2:1) to precipitate the insoluble portion, which was subjected to silica gel column chromatography to provide three steroidal fractions (fr.a, fr.b, fr.c), each of which gave a single spot on a thin-layer chromatogram (TLC). At this stage, the TLC analysis of the above-obtained crystals showed that the crystals contained components moving on TLC with the same *Rf* value as the compounds contained in fr.b. The fr.c was further subjected to separation by high-performance liquid chromatography (HPLC) to provide two 4-methylene



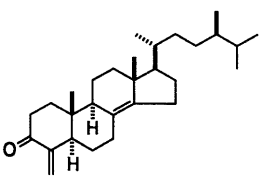
theonellasterol (**1**)



conicasterol (**2**)



theonellasterone (**3**)



conicasterone (**4**)

Chart 1

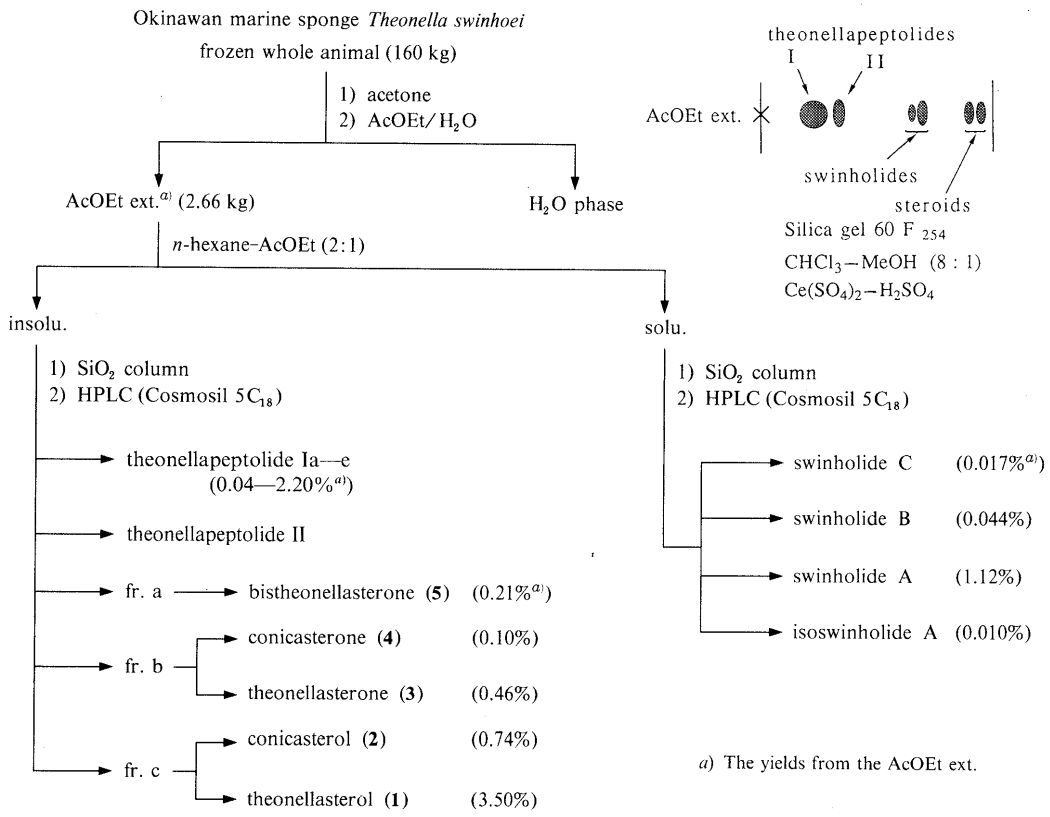


Fig. 1

sterols being identical with theonellasterol (**1**) and conicasterol (**2**), which were previously isolated from the Red Sea marine sponge of the same species by Djerassi and his group.[15] The HPLC separation of fr.b afforded two new 3-keto-4-methylene steroids now designated theonellasterone (**3**) and conicasterone (**4**).

The major compound, named theonellasterone (**3**), showed absorption bands due to a conjugated enone moiety [204 nm ($\varepsilon = 14000$) and 1685, 1605 cm^{-1}] in its ultraviolet (UV) and infrared (IR) spectra. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** showed signals assignable to one exomethylene moiety [δ 5.82, 5.07 (both 1H, s)], two singlet methyl [δ 0.87, 0.76 (both 3H)], three doublet methyl [δ 0.95, 0.84, 0.81 (each 3H)], and one triplet methyl [δ 0.86 (3H)] groups. These spectral properties have led us to presume that theonellasterone (**3**) is a 3-keto analogue of co-occurring theonellasterol (**1**). In order to verify this presumption, theonellasterone (**3**) was treated with sodium borohydride ($NaBH_4$) in the presence of ceric chloride ($CeCl_3 \cdot 7H_2O$) to furnish theonellasterol (**1**) in quantitative yield. Furthermore, when **1** was treated with pyridinium chlorochromate (PCC), **3** was recovered quantitatively. Consequently, the structure of theonellasterone (**3**) has been determined to be 4-methyl-24α-ethyl-5α-cholesta-4(30),8(14)-dien-3-one as shown.

The minor compound, named conicasterone (**4**), also showed UV and IR absorption bands [200 nm ($\varepsilon = 14000$) and 1685, 1605 cm^{-1}] which indicated the presence of a conjugated enone moiety as seen in theonellasterone (**3**). The ^{1}H-NMR spectrum of **4** showed the signals assignable to one exomethylene moiety [δ 5.82, 5.07 (both 1H, s)], two tertiary methyl groups [δ 0.87, 0.76 (both 3H)] and four secondary methyl [δ 0.97, 0.86, 0.81, 0.79 (each 3H)] groups. Furthermore, oxidation of conicasterol (**2**) with PCC gave **4** in quantitative yield. Therefore, the structure of conicasterone (**4**) has been determined as 4,24β-dimethyl-5α-cholesta-4(29),8(14)-dien-3-one.

After characterization of four steroids, theonellasterol (**1**), conicasterol (**2**), theonellasterone (**3**), and conicasterone (**4**), we next analyzed the above-described crystals which were obtained as the deposit in the tissue of the marine sponge. The HPLC analysis of the crystals showed that these crystals were a mixture of theonellasterone (**3**) and conicasterone (**4**) and did not contain sterols.

Configurations of the 24-ethyl residue in theonellasterol (**1**) and the 24-methyl residue in conicasterol (**2**) were determined by Djerassi and his group on the basis of detailed comparisons of the ^{1}H-NMR chemical shifts of the methyl groups in the side chains of these sterols.[15] However, it seemed a rather difficult task to determine definitely the 24-ethyl configuration in this manner, since the ^{1}H-NMR spectra of 24α-(*S*)-ethyl and 24β-(*R*)-ethyl sterols are very similar.[16] In order to obtain supplementary evidence for assigning the configuration of the ethyl residue in the side chains of theonellasterol (**1**) and theonellasterone (**3**), we have carried out the following study. It was presumed that 24α-ethyl and 24β-ethyl sterols each take distinctive side chain conformations in solution regardless of the skeletal structures. So, these side chains of sterols may contribute independently to the optical rotatory properties.[17] It has been considered therefore that the Hudson rule[18] may be applicable to assign the configuration of the 24-ethyl residue in the side chains of these steroids in comparison with the 24-methylsteroids. The molecular rotations $[M]_D$ of related

TABLE I. Comparisons of $[M]_D$ Values between 24β-Methylsteroids and 24α- and 24β-Ethylsteroids

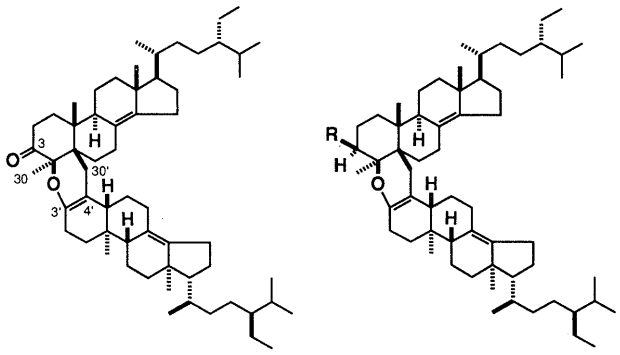
	$[\alpha]_D$ ($CHCl_3$)	$[M]_D$		$[\alpha]_D$ ($CHCl_3$)	$[M]_D$		$[\alpha]_D$ ($CHCl_3$)	$[M]_D$		$[\alpha]_D$ ($CHCl_3$)	$[M]_D$
Campesterol (24β-methyl)	−33°	−136.6°	Campesterol (24β-methyl)	−33°	−136.6°	Conicasterone (**4**) (24β-methyl)	+42.1°	+178.5°	Conicasterol (**2**) (24β-methyl)	+57.5°	+245.0°
β-Sitosterol (24β-ethyl)	−37°	−153.2°	γ-Sitosterol (24α-ethyl)	−43°	−178.0°	Theonellasterone (**3**) (24α-ethyl)	+34.3°	+145.4°	Theonellasterol (**1**) (24α-ethyl)	+48.7°	+207.5°
$\Delta[M]_D$		+16.6°	$\Delta[M]_D$		+41.4°	$\Delta[M]_D$		+33.1°	$\Delta[M]_D$		+37.5°

steroids were examined and the results were as shown in Table I. The $\Delta[M]_D$ values between conicasterol (**2**) and theonellasterol (**1**) (+37.5°) and between conicasterone (**4**) and theonellasterone (**3**) (+33.1°) are very close to the $\Delta[M]_D$ value between campesterol[19,20] (=24β-methylcholest-5-en-3-ol) and γ-sitosterol[20,21] (=24α-ethylcholest-5-en-3-ol) (+41.4°), but are markedly different from the $\Delta[M]_D$ value between campesterol and β-sitosterol[20,22] (=24β-ethylcholest-5-en-3-ol) (+16.6°). It follows therefore that comparison of the molecular rotation values also supported the 24α-ethyl (*S*) configuration in theonellasterol (**1**) and theonellasterone (**3**). In other words, application of the Hudson rule is a reliable method for judging the 24-ethyl configuration in the steroid side chain provided that the 24-methyl counterparts, or counterparts with an ordinary cholestane side chain, are available.

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of fr.a (Fig. 1) showed the signals of one ketonic carbon [δ_C 209.5 (s)], six olefinic carbons [δ_C 146.1, 143.5, 142.2, 126.4, 125.3, 102.8 (each s)], and one quaternary carbon bearing an oxygen function [δ_C 83.9 (s)] together with rather complicated signals attributable to the side chain carbons. These spectral properties have led us to presume that fr.a contains a mixture of dimeric steroids, probably formed through Diels–Alder type dimerizations of theonellasterone (**3**) and/or conicasterone (**4**). Since further separation of fr.a into components was not successful, the following device was employed. Thus, $NaBH_4$ treatment of fr.a proceeded smoothly to provide a mixture of dihydro derivatives, which was further subjected to HPLC separation to isolate the major dihydro derivative **6**. The ^1H-NMR spectrum of **6** showed the signal of one carbinyl proton at δ 3.66 (1H, dd) assignable to 3α-H.

On the other hand, a solution of theonellasterone (**3**) in chloroform was concentrated under reduced pressure and warmed at 50 °C for 2 h to afford a dimerization product named bistheonellasterone (**5**) in excellent yield (92%). Bistheonellasterone (**5**) thus obtained was shown to move on TLC with the same *Rf* value as that of fr.a. Furthermore, $NaBH_4$ reduction of **5** quantitatively furnished a dihydro derivative, which was found to be identical with the major component **6** obtained above by $NaBH_4$ treatment of fr.a. Thus, it has become clear that bistheonellasterone (**5**) itself must be the major component of fr.a, which also contains other dimeric steroids probably formed from theonellasterone (**3**) and/or conicasterone (**4**) as minor components.

In order to define the stereostructure of bistheonellasterone (**5**), the following investigation was carried out. First, the correlation spectroscopy *via* long-range coupling (COLOC) spectrum of **6** showed the long-range correlation signal between the 3α-proton (δ 3.66) and C-4 carbon [δ_C 80.6 (s)]. In addition, in the ^1H-detected multiple-



bistheonellasterone (**5**)

6 : R = OH
7 : R = OAc

Chart 2

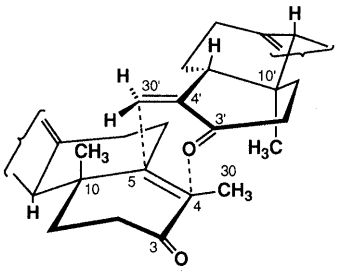


Fig. 2

bond heteronuclear multiple quantum coherence (HMQC) spectrum of the monoacetate **7**, which was prepared by acetylation of **6**, the long-range correlation between the 3α-proton (δ 4.96) and C-4 carbon [δ_C 77.8 (s)] was also observed. When a solution of **5** in benzene–dioxane was treated with concentrated aqueous hydrogen chloride, an α-hydroxy ketonic compound [presumably formulated as (A) derived through ring opening of the dihydropyran moiety] was formed. The compound was then subjected to oxidation with lead tetraacetate [$Pb(OAc)_4$] in methanol[23] to yield a diketo-ester **8**. The ^1H-NMR and ^{13}C-NMR spectra of **8** showed signals ascribable to one methoxycarbonyl group [δ 3.76 (3H, s); δ_C 174.3 (C-3), 56.9 (OMe)], one methyl ketone moiety and one cyclic ketone moiety [δ 1.58 (3H, s); δ_C 214.1, 213.3 (C-4, C-3′)]. Thus, the plain structures of **5** and **6** have been elucidated as shown.

Next, the stereochemistry of bistheonellasterone (**5**) was investigated. The ^1H-NMR coupling constants (dd, J = 11.9, 3.7 Hz) of 3-H of **6** are consistent with α-axial configuration. So, it was presumed that hydride attacked from the less-hindered α-side of the 3-ketone moiety of **5**. The dihydro derivative **6** was rather unstable and, on treatment with *p*-toluenesulfonic acid at room temperature, it was readily converted to a cyclic ketal **9**. The absolute configuration at

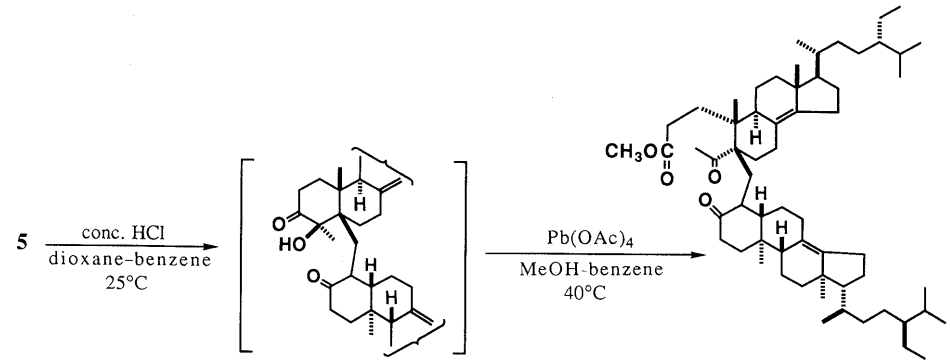


Chart 3

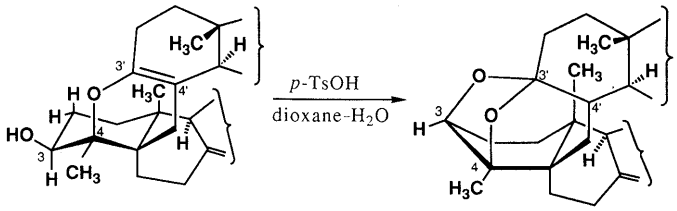


Chart 4

C-3 of **6** was determined to be *S* by means of the modified Horeau's method.[24] Based on the accumulated evidence, the stereostructures of bistheonellasterone (**5**) and **6** have been determined as shown. Interestingly, the intermolecular Diels–Alder cycloaddition of theonellasterone (**3**), both biologically and chemically, has occurred regio- and stereo-specifically to afford bistheonellasterone (**5**) as shown in Fig. 2, which is reminiscent of the regioselective Diels–Alder cycloaddition of acrolein.[25]

Experimental

The instruments used to obtain physical data and experimental conditions for chromatography were the same as described in our preceding paper.[8]

Isolation of Theonellasterol (1), Conicasterol (2), Theonellasterone (3), and Conicasterone (4) The fresh marine sponge *Theonella swinhoei* (1.5 kg), which was collected at Hedo Cape, Okinawa in May, was extracted with acetone at room temperature, and the extract was concentrated under reduced pressure to give an aqueous suspension, which was extracted with AcOEt. The AcOEt-soluble portion was evaporated under reduced pressure to give the extract (24 g). The extract (5 g) was subjected to column chromatography (Kieselgel 60, *n*-hexane–AcOEt) to furnish fr.a (24 mg), fr.b (125 mg), and fr.c (270 mg). Fraction c (270 mg) was further subjected to HPLC [Shimpack PREP ODS, $CHCl_3$–MeOH–CH_3CN–H_2O (25 : 60 : 10 : 5)] to isolate theonellasterol (**1**) (175 mg) and conicasterol (**2**) (37 mg). Theonellasterol (**1**) and conicasterol (**2**) were shown to be identical with those obtained from the Red Sea marine sponge of the same species by Djerassi *et al.*[15] by ^{1}H- and ^{13}C-NMR comparisons. Theonellasterol (**1**): $[\alpha]_D$ +48.7° (c = 1.6, $CHCl_3$, 26 °C). Conicasterol (**2**): $[\alpha]_D$ +57.5° (c = 0.9, $CHCl_3$, 26 °C).

Fraction b (125 mg) was further separated by HPLC [Shimpack PREP ODS, $CHCl_3$–MeOH–CH_3CN–H_2O (30 : 50 : 10 : 5)] to furnish theonellasterone (**3**) (23 mg) and conicasterone (**4**) (5 mg).

Theonellasterone (**3**): Colorless needles, mp 99—101 °C (CH_3CN). $[\alpha]_D$ +34.3° (c = 1.05, $CHCl_3$, 26 °C). UV $\lambda_{max}^{n\text{-hexane}}$ nm (ε): 204 (14000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2960, 2870, 1685, 1605, 1460, 1375. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 5.82, 5.07 (both 1H, s, 30-H_2), 0.95 (3H, d, J = 6.7 Hz, 20-Me), 0.87 (3H, s, 13-Me), 0.86 (3H, t, J = 7.3 Hz, 28-Me), 0.84, 0.81 (both 3H, d, J = 7.0 Hz, 25-Me), 0.76 (3H, s, 10-Me). ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): 203.0 (s, C-3), 149.4 (s, C-4), 143.7 (s, C-14), 124.9 (s, C-8), 118.3 (t, C-30), 56.8 (d, C-17), 49.7 (d, C-5), 48.5 (d, C-9), 46.2 (d, C-24), 42.8 (s, C-13), 38.2 (s, C-10), 37.3 (t, C-12), 37.1 (t, C-22), 35.6 (t, C-2), 34.9 (d, C-20), 33.8 (t, C-1), 29.1 (t, C-7), 29.1 (d, C-25), 27.1 (t, C-6), 26.3 (t, C-16), 25.9 (t, C-23), 24.8 (t, C-15), 23.1 (t, C-28), 20.4 (t, C-11), 19.6 (q, C-21), 19.3, 19.1 (both q, C-26, 27), 18.3 (q, C-18), 12.7 (q, C-29), 12.4 (q, C-19). High-resolution mass spectrum (HR-MS): Obsd: m/z 424.626. Calcd for $C_{30}H_{48}O$: 424.623 (M^+).

Conicasterone (**4**): Colorless needles, mp 91—93 °C (CH_3CN). $[\alpha]_D$ +42.1° (c = 1.25, $CHCl_3$, 26 °C). UV $\lambda_{max}^{n\text{-hexane}}$ nm (ε): 200 (14000). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 2860, 1685, 1605, 1450, 1375. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 5.82, 5.07 (both 1H, s, 29-H_2), 0.97 (3H, d, J = 6.4 Hz, 20-Me), 0.87 (3H, s, 13-Me), 0.86, 0.81 (both 3H, d, J = 7.0 Hz, 25-Me), 0.79 (3H, d, J = 6.7 Hz, 24-Me), 0.76 (3H, s, 10-Me). ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): 203.2 (s, C-3), 149.5 (s, C-4), 143.8 (s, C-14), 124.9 (s, C-8), 118.4 (t, C-29), 56.9 (d, C-17), 49.7 (d, C-5), 48.6 (d, C-9), 42.8 (s, C-13), 39.0 (d, C-24), 38.3 (s, C-10), 37.3 (t, C-12), 37.1 (t, C-22), 35.6 (t, C-2), 34.6 (d, C-20), 33.6 (t, C-1), 32.5 (d, C-25), 30.3 (t, C-23), 29.1 (t, C-7), 27.1 (t, C-6), 25.9 (t, C-16), 24.8 (t, C-15), 20.4 (t, C-11), 20.3 (q, C-26), 19.2 (q, C-21), 18.3 (q, C-18), 18.3 (q, C-27), 15.5 (q, C-28), 12.8 (q, C-19). HR-MS: Obsd: m/z 410.355. Calcd for $C_{29}H_{46}O$: 410.355 (M^+).

Fraction a: ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 2.70 (ddd, J = 4.9, 13.4, 13.4 Hz), 2.45 (m), 1.51 (s), 0.96—0.82 (m), 0.67 (s). ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): 209.5 (s), 146.1 (s), 143.5 (s), 142.2 (s), 126.4 (s), 125.3 (s), 102.8 (s), 83.9 (s), 57.3 (t), 57.0 (t), 51.3 (t), 48.2 (t), 46.3 (t), 46.1 (t), 42.8 (s), 38.4 (s), 37.6 (s), 37.2, 37.0, 35.1, 35.0, 34.9, 33.9, 29.8, 29.6, 29.2, 27.1, 27.0, 26.6, 26.0, 25.8, 25.0, 24.8, 23.2, 22.0, 20.3, 20.3, 19.9, 19.6, 19.3, 19.2, 18.7, 18.6, 18.3, 15.5, 14.8, 13.1, 12.4.

Reduction of Theonellasterone (3) A solution of **3** (80 mg) in MeOH–benzene (2 : 1, 10 ml) was treated with $NaBH_4$ (14 mg) and $CeCl_3 \cdot 7H_2O$ (120 mg) and the whole was stirred under an N_2 atmosphere at 25 °C for 20 min. The reaction mixture was partitioned into an AcOEt–H_2O mixture and then the AcOEt-soluble portion was washed with brine and dried over $MgSO_4$. Removal of the solvent from the AcOEt-soluble portion under reduced pressure gave a product, which was purified by SiO_2 column chromatography (*n*-hexane–AcOEt) to furnish theonellasterol (**1**) (72 mg).

Oxidation of Theonellasterol (1) A solution of **1** (141 mg) in CH_2Cl_2 (5 ml) was treated with PCC (214 mg) and the whole was stirred under an N_2 atmosphere at 25 °C for 30 min. The reaction mixture was poured into water and extracted with CH_2Cl_2. The CH_2Cl_2-soluble portion was washed with brine and dried over $MgSO_4$. Removal of the solvent from the CH_2Cl_2-soluble portion under reduced pressure gave a product, which was purified by SiO_2 column chromatography (*n*-hexane–AcOEt) to furnish theonellasterone (**3**) (132 mg).

Oxidation of Conicasterol (2) A solution of **2** (30 mg) in CH_2Cl_2 (3 ml) was treated with PCC (48 mg) and the whole was stirred under an N_2 atmosphere at 25 °C for 30 min. Work-up of the reaction mixture as described above gave a product, which was purified by SiO_2 column chromatography to furnish conicasterone (**4**) (28 mg).

Reduction of Fr.a A solution of fr.a (50 mg) in ethanol–benzene (2 : 1, 10 ml) was treated with $NaBH_4$ (25 mg) and the whole was stirred under an N_2 atmosphere at 25 °C for 30 min. Work-up of the reaction mixture as described above gave a product, which was subjected to SiO_2 column chromatography (*n*-hexane–benzene) and HPLC [Develosil ODS-5, $CHCl_3$–MeOH–H_2O (6 : 5 : 1)] to furnish **6** (22 mg).

6: A white powder, $[\alpha]_D$ +7.3° (c = 1.24, $CHCl_3$, 26 °C). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580, 2940, 2870, 1685, 1455, 1370, 1160. ^{1}H-NMR (500 MHz,

$CDCl_3$, δ): 3.66 (1H, dd, J=11.9, 3.7 Hz, H-3), 0.94 (6H, d, J=6.3 Hz, 20,20′-Me), 0.87, 0.86 (both 3H, t, J=7.3 Hz, 28,28′-Me), 0.84 (9H, s, 4,13,13′-Me), 0.85 (6H, d, J=7.3 Hz, 25,25′-Me), 0.82 (6H, d, J=7.1 Hz, 25,25′-Me), 0.79 (3H, s, 10-Me), 0.67 (3H, s, 10′-Me). ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): 146.9 (s, C-3′), 142.6, 142.5 (both s, C-14, 14′), 126.4, 126.0 (both s, C-8, 8′), 104.6 (s, C-4′), 80.6 (s, C-4), 80.4 (d, C-3), 57.1, 57.0 (both d, C-17, 17′), 51.8 (d, C-5′), 48.2 (d, C-9,9′), 47.2, 46.4 (both d, C-24, 24′), 42.9 (s, C-13, 13′), 42.8 (s, C-10), 39.4 (s, C-5), 37.5 (3C, t, C-12, 12′, 30′), 37.4, 37.1 (both t, C-22, 22′), 37.1 (s, C-10′), 35.1, 35.0 (both d, C-20, 20′), 33.9, 33.8 (both t, C-1, 1′), 30.3, 29.9 (both t, C-7, 7′), 29.3 (q, C-30), 27.2, 27.1 (both t, C-6, 6′), 26.6, 26.4 (both t, C-16, 16′), 25.9, 25.8 (both t, C-23, 23′), 25.6, 24.2 (both t, C-15, 15′), 23.3, 22.4 (both t, C-28,28′), 20.5, 20.3 (both t, C-11, 11′), 19.7 (q, C-21, 21′), 19.6 (q, C-26, 26′), 19.4, 19.2 (both q, C-27, 27′), 18.7, 18.3 (both q, C-18, 18′), 14.8 (q, C-29, 29′), 13.3, 12.4 (both q, C-19,19′). Fast atom bombardment (FAB)-MS: m/z: 851 $(M+H)^+$.

Conversion from Theonellasterone (3) to Bistheonellasterone (5) A solution of **3** (21 mg) in $CHCl_3$ (3 ml) was concentrated under reduced pressure and warmed at 50 °C for 2 h. The residue was subjected to SiO_2 column chromatography (*n*-hexane–benzene) to furnish **5** (11 mg) and **3** (recovered, 9 mg).

Bistheonellasterone (**5**): A white powder, $[\alpha]_D$ +1.6° (c=1.45, $CHCl_3$, 26 °C). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 2870, 1720, 1685, 1460, 1375, 1160, 1050. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 2.69 (1H, ddd, J=13.7, 13.7, 4.9 Hz, H-2 (ax.)), 2.25 (1H, m, H-2 (eq.)), 1.99 (1H, m, H-1 (eq.)), 1.56 (1H, m, H-1 (ax.)), 1.03 (3H, s, 4-Me), 0.94 (6H, d, J=6.1 Hz, 20,20′-Me), 0.86 (6H, t, J=6.4 Hz, 28,28′-Me), 0.84 (6H, d, J=7.4 Hz, 25, 25′-Me), 0.84 (6H, s, 13,13′-Me), 0.81 (6H, d, J=7.1 Hz, 25,25′-Me), 0.65 (6H, s, 10,10′-Me). ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): 209.8 (s, C-3), 146.1 (s, C-3′), 143.6, 142.3 (both s, C-14, 14′), 126.4, 125.3 (both s, C-8,8′), 102.9 (s, C-4′), 83.9 (s, C-4), 57.3, 57.0 (both d, C-17, 17′), 5.13 (d, C-5′), 48.2 (d, C-9, 9′), 46.3, 46.1 (both d, C-24, 24′), 42.9 (s, C-13, 13′), 39.7 (t, C-30′), 38.4 (s, C-10, 5), 37.6 (t, C-12, 12′), 37.3 (t, C-22, 22′), 37.1 (s, C-10′), 35.0 (d, C-20, 20′), 33.9 (t, C-1, 1′), 29.8, 29.7 (both t, C-7, 7′), 29.2 (q, C-30), 27.2, 27.1 (both t, C-6, 6′), 26.6 (t, C-16, 16′), 26.0 (t, C-23, 23′), 25.0, 24.9 (both t, C-15,15′), 23.3, 22.0 (both t, C-28, 28′), 20.3, 19.9 (both t, C-11, 11′), 19.7 (q, C-21, 21′), 19.4 (q, C-26, 26′), 19.2 (q, C-27, 27′), 18.6, 18.4 (both q, C-18, 18′), 14.8 (q, C-29, 29′), 13.0, 12.4 (both q, C-19, 19′). FAB-MS: m/z 849 $(M+H)^+$. *Anal.* Calcd for $C_{60}H_{96}O_2 \cdot 1/2H_2O$: C, 83.94; H, 11.39. Found: C, 84.16; H, 11.35.

Reduction of Bistheonellasterone (5) Giving 6 A solution of **5** (20 mg) in ethanol–benzene (2 : 1, 10 ml) was treated with $NaBH_4$ (10 mg) and the whole was stirred under an N_2 atmosphere at 25 °C for 3 h. Work-up of the reaction mixture as described above gave a product, which was purified by SiO_2 column chromatography (*n*-hexane–benzene) to furnish **6** (18 mg).

Acetylation of 6 Giving 7 A solution of **6** (16 mg) in pyridine (2 ml) was treated with acetic anhydride (1.5 ml) and the whole was stirred at 25 °C for 2 hr. The reaction mixture was poured into water and extracted with AcOEt. The AcOEt extract was washed with brine and dried over $MgSO_4$. Removal of the solvent under reduced pressure from the AcOEt solution furnished a product, which was purified by SiO_2 column chromatography (*n*-hexane–benzene) to give **7** (14 mg).

7: A white powder, $[\alpha]_D$ −8.19° (c=0.85, $CHCl_3$, 26 °C). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 2865, 1725, 1685, 1460, 1375. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 4.96 (1H, br d, J=*ca.* 7.9 Hz, H-3), 1.98 (3H, s, CH_3CO), 0.84 (6H, s, 13,13′-Me), 0.78 (6H, s, 10,4-Me), 0.65 (3H, s, 10′-Me). ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): 170.2 (s, CO), 146.3 (s, C-3′), 142.8, 142.5 (both s, C-14, 14′), 126.4, 125.9 (both s, C-8, 8′), 102.9 (s, C-4′), 81.2 (d, C-3), 77.8 (s, C-4), 57.1, 57.0 (both d, C-17, 17′), 51.6 (d, C-5′), 48.4 (d, C-9, 9′), 47.4, 46.4 (both d, C-24, 24′), 42.9 (s, C-13, 13′), 42.8 (s, C-10), 38.8 (s, C-5), 37.5 (3C, t, C-12, 12′, 30′), 37.2 (s, C-10′), 37.0 (t, C-22, 22′), 35.1, 35.0 (both d, C-20, 20′), 34.1, 34.0 (both t, C-1, 1′), 30.1, 30.0 (both t, C-7, 7′), 29.3 (q, C-30), 27.2, 27.1 (both t, C-6, 6′), 26.6, 26.0 (both t, C-16, 16′), 25.9, 25.8 (both t, C-23, 23′), 25.4, 25.3 (both t, C-15, 15′), 23.3, 23.1 (both t, C-28, 28′), 21.4 (q, CH_3CO), 20.3, 20.0 (both t, C-11, 11′), 19.7 (q, C-26, 26′), 19.4, 19.3 (both q, C-27, 27′), 18.6, 18.3 (both q, C-18, 18′), 14.7 (q, C-29, 29′), 13.1, 12.4 (both q, C-19, 19′).

Acidic Treatment Followed by Oxidation of 5 Giving 8 A solution of **5** (60 mg) in benzene–dioxane (5 : 1, 6 ml) was treated with concentrated HCl (0.1 ml) and the whole was stirred at 25 °C for 1 h. The solvent was evaporated from the reaction mixture under reduced pressure to give a residue, which was dissolved in benzene–MeOH (5 : 1, 6 ml), and the solution was treated with $Pb(OAc)_4$ (30 mg). The reaction mixture was stirred at 40 °C for 3 h and poured into a benzene–water mixture. The benzene-soluble portion was taken, washed with brine and dried over $MgSO_4$. Removal of the solvent from the benzene-soluble portion under reduced pressure gave a product, which was purified by SiO_2 column chromatography (*n*-hexane–benzene–AcOEt) to furnish **8** (11 mg).

8: A white powder. $[\alpha]_D$ +16.2° (c=0.2, $CHCl_3$, 20 °C). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2955, 2930, 1720, 1710, 1458, 1370. ^{1}H-NMR (270 MHz, $CDCl_3$, δ): 3.76 (3H, s), 1.58 (3H, s). ^{13}C-NMR (67.5 MHz, $CDCl_3$, δ_C): 214.1, 213.3 (C-4, C-3′), 174.3 (C-3), 144.0, 143.4 (C-14, 14′), 125.1, 124.6 (C-8, 8′), 56.9 (OMe). HRFAB-MS: Obsd: m/z 903.780. Calcd for $C_{61}H_{100}O_4$: 903.778 $(M+Li)^+$.

Acidic Treatment of 6 Giving 9 A solution of **5** (30 mg) in dioxane–H_2O (10 : 1, 5.5 ml) was treated with *p*-toluenesulfonic acid (*p*-TsOH·H_2O) (1 mg) and the whole was stirred at 25 °C for 30 min. The reaction mixture was partitioned into a $CHCl_3$–H_2O mixture. The $CHCl_3$-soluble portion was taken, washed with brine and dried over $MgSO_4$. Removal of the solvent from the $CHCl_3$ solution furnished a product, which was subjected to SiO_2 column chromatography (*n*-hexane–AcOEt) to give **9** (23 mg).

9: A white powder, $[\alpha]_D$ −2.2° (c=0.91, $CHCl_3$, 26 °C). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 2835, 1460, 1380. ^{1}H-NMR (500 MHz, $CDCl_3$, δ): 3.49 (1H, br d, J=*ca.* 10.7 Hz, H-3), 0.93 (6H, s, 13,13′-Me), 0.78 (6H, s, 10,10′-Me). ^{13}C-NMR (125 MHz, $CDCl_3$, δ_C): 143.3, 142.3 (both s, C-14, 14′), 126.4, 125.6 (both s, C-8, 8′), 109.7 (s, C-3′), 86.1 (d, C-3), 82.1 (s, C-4), 56.9 (d), 53.5 (d), 52.2 (d), 48.9 (d), 48.2 (d), 46.2 (d), 42.8 (d), 42.8 (s), 41.2 (d), 39.0 (d), 37.7 (t), 37.5 (t), 37.4 (d), 37.1 (t), 35.0 (t), 34.9 (d), 33.9 (t), 33.8 (t), 31.2 (t), 29.6 (t), 29.2 (t), 29.0 (d), 27.2 (t), 27.1 (t), 26.5 (t), 26.3 (t), 26.2 (t), 26.0 (t), 25.9 (t), 24.1 (t), 23.1 (t), 22.9 (t), 21.9 (t), 20.1 (t), 19.9 (t), 19.6 (q), 19.3 (q), 19.1 (q), 18.8 (q), 18.3 (q), 16.2 (q), 13.0 (q), 12.4 (q). FAB-MS: m/z 849 $(M-H)^+$. HRFAB-MS: Obsd: m/z 849.747; 850.755. Calcd for $C_{60}H_{97}O_2$: 849.745 $(M-H)^+$; $C_{60}H_{98}O_2$: 850.753 (M^+).

Application of Horeau's Method to 6 A solution of **6** (3 mg, 4 μmol) in pyridine (36 μl) was treated with (±)-α-phenylbutyric anhydride (3.6 mg, 11 μmol), and the whole was kept in a sealed vial at 40 °C for 3 h. (+)-(*R*)-α-Phenylethanolamine (10 μl) was added to the reaction mixture and the whole was mixed thoroughly by agitation for 15 min. The reaction mixture was diluted with AcOEt (400 μl) and then analyzed by gas liquid chromatography (GLC) [column: OV-17 FFS (Scot) capillary column 0.32 mm × 50 m; column temperature, 150 °C]. A parallel reaction was carried out with cyclohexanol (18 μmol) in a similar manner. The relative proportions of the amides of (−)-(*R*)- and (+)-(*S*)-α-phenylbutyric acid were measured and the corresponding value obtained by the reaction with cyclohexanol was subtracted. The increment of the amide of (−)-(*R*)-α-phenylbutylic acid was 6%.

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