40 cc/min). Bicyclo[2.2.1]hepta-2,5-diene (retention time, 6.75 min) was collected (9.4% yield) and confirmed by comparison with an authentic sample (IR in CCl_4).

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Supplementary Material Available: Aromatic and benzyl ^{13}C NMR chemical shifts and ^{13}C–^{31}P coupling constants of tetracyclic derivatives; ^{13}C–H coupling constants in oxide **1a**; lanthanide-induced ^{13}C and ^{1}H NMR chemical shift gradients of **1a** and **1b** (3 pages). Ordering information is given on any current masthead page.

Hyrtiosal, a New Sesterterpenoid with a Novel Carbon Skeleton from the Okinawan Marine Sponge *Hyrtios erectus*

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Hyrtiosal, a new sesterterpenoid possessing a novel rearranged tricarbocyclic skeleton, was isolated from the Okinawan marine sponge, *Hyrtios erectus* (Keller, 1891). This compound was shown to inhibit the proliferation of KB cells in vitro, and its structure was determined by chemical and spectral methods including two-dimensional ^{13}C–^{1}H long-range correlations. The relative stereochemistry was determined based on two-dimensional NOE correlations. A possible biosynthesis of hyrtiosal is briefly discussed.

Marine sponges are recognized as a rich source of structurally unique and biologically active terpenoids.[1,2] In the course of our investigation[3] on biologically active substances from Okinawan marine animals, we isolated a new sesterterpenoid, hyrtiosal (1), from the Okinawan sponge, *Hyrtios erectus* (Keller, 1891) (also called *Heteronema erecta*). The compound exhibited in vitro antiproliferative activity against KB cells with an IC_{50} of 3–10 μg/mL. From *H. erecta* which inhabits the Australian Great Barrier Reef, a scalarane type sesterterpenoid with a tetracarbocyclic skeleton, called heteronemin, was isolated by Kazlauskas et al.[4] Several scalarane type sesterterpenoids have also been isolated from Tongan *Hyrtios erecta* by Crews et al.[5] No scalarane type sesterterpenoid could be isolated from the present Okinawan sponge, but a new class of sesterterpenoid possessing a novel rearranged tricarbocyclic skeleton was obtained. Elucidation was made of the structure of hyrtiosal (1) on the basis of results from spectroscopic analysis and chemical reactions.

Wet specimens of *H. erectus*[6] (2.2 kg), obtained from the coral reef of Ishigaki Island (Okinawa, Japan), were extracted with methanol. The ethyl acetate soluble portion (3.7 g) of the methanol extract was chromatographed on a silica gel column. The fraction obtained by elution with hexane–ethyl acetate (1:1) was further purified by repeated silica gel column chromatography, followed by preparative TLC to give hyrtiosal (1) as colorless needles (21 mg, mp 119–121 °C).

The molecular formula $C_{25}H_{38}O_3$ of 1 was determined based on HRMS measurement. All 25 carbons were appeared in the ^{13}C NMR spectra measured in both $CDCl_3$ and C_6D_6 solutions, and DEPT indicated the presence of five methyls, seven methylenes, four sp^3 methines, three sp^2 methines, four sp^3 quaternary carbons, and two sp^2 quaternary carbons. Table I presents ^{13}C and ^{1}H NMR correlations found through examination of the two-dimensional ^{13}C–^{1}H COSY spectrum. IR, ^{1}H NMR ($CDCl_3$), and ^{13}C NMR ($CDCl_3$) spectra showed the presence of a formyl group (IR 1708 cm^{-1}, δ_H 9.45 (s), δ_C 205.7), secondary hydroxy group (IR 3547 cm^{-1}, δ_H 4.42 (dd, J = 6.0, 7.4 Hz), δ_C 64.2), and monosubstituted furan moiety (δ_H 6.37 (t, J = 1.1 Hz), 7.36 (s), 7.37 (br s), δ_C 109.5, 129.3, 139.8, 143.2). Partial structures of CH_2CH_2 (from C-1 to C-2), $CHCH_2CH_2$ (from C-5 to C-7), $CHCH_2$ (from C-9 to C-11) and $CHCH_2CHOH$ (from C-14 to C-16) were surmised based on analysis of ^{1}H coupling constants (Table I) and confirmed by two-dimensional ^{1}H–^{1}H COSY measurement.

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(6) The sponge was identified by Prof. R. W. M. van Soest, Institute of Taxonomic Zoology, University of Amsterdam. The specimens are on deposit in his collection (registered number: ZMA POR. 9434).

0022-3263/92/1957-0522$03.00/0

Table I. ^{13}C and ^{1}H NMR Data for 1

no.	^{13}C (in $CDCl_3$)[a]	^{13}C (in C_6D_6)[a]	^{1}H (in $CDCl_3$)[b]	^{1}H (in C_6D_6)[b]
1	40.2 CH_2	40.2 CH_2	overlapped	0.78 (dt, 3.6, 12.5, Hα) 1.35 (br dd, 3.1, 12.5, Hβ)
2	18.8 CH_2	18.6 CH_2	overlapped	1.44 (m), 1.65 (m)
3	42.4 CH_2	42.6 CH_2	overlapped	1.22 (dt, 4.5, 13.0, Hα) 1.48 (m, Hβ)
4	33.1 C	33.1 C	–	–
5	57.4 CH	57.0 CH	0.85 (m)	0.70 (dd, 2.4, 12.5)
6	18.3 CH_2	19.0 CH_2	1.40 (m), 1.60 (m)	1.33 (dq, 3.0, 12.5, Hβ) 1.53 (qd, 2.4, 12.5, Hα)
7	40.3 CH_2	40.1 CH_2	1.13 (m, Hα) 1.72 (td, 3.2, 12.5, Hβ)	1.05 (dt, 3.8, 12.5, Hα) 1.65 (m, Hβ)
8	44.5 C	44.5 C	–	–
9	60.4 CH	60.3 CH	1.07 (dd, 6.2, 13.8)	0.97 (dd, 6.3, 15.1)
10	36.8 C	36.6 C	–	–
11	33.66 CH_2	33.7 CH_2	1.40 (m, Hβ) 1.89 (dd, 6.3, 13.1, Hα)	1.18 (dd, 12.8, 15.1, Hβ) 1.72 (dd, 6.3, 12.8, Hα)
12	205.7 CH	204.5 CH	9.45 (s)	9.43 (s)
13	52.8 C	52.6 C	–	–
14	48.1 CH	49.5 CH	1.98 (dd, 6.6, 7.9)	2.15 (dd, 3.6, 10.6)
15	33.69 CH_2	34.2 CH_2	1.62 (m)	1.68 (m)
16	64.2 CH	64.4 CH	4.42 (dd, 6.0, 7.4)	4.51 (dd, 3.7, 9.4)
17	129.3 C	130.4 C	–	–
18	109.5 CH	109.9 CH	6.37 (t, 1.1)	6.37 (br s)
19	143.2 CH	143.2 CH	7.36 (s) or 7.37 (br s)	7.22 (t, 1.7)
20	33.5 CH_3	33.5 CH_3	0.845 (s)	0.95 (s)
21	21.2 CH_3	21.4 CH_3	0.82 (s)	0.93 (s)
22	15.7 CH_3	15.7 CH_3	0.86 (s)	0.84 (s)
23	16.5 CH_3	16.6 CH_3	0.851 (s)	0.74 (s)
24	19.1 CH_3	19.1 CH_3	1.18 (s)	1.09 (s)
25	139.8 CH	139.0 CH	7.36 (s) or 7.37 (br s)	7.34 (br s)

[a] ^{13}C NMR spectra were recorded at 125 MHz in $CDCl_3$ and C_6D_6 solutions, respectively. Numbers of attached protons were determined by DEPT experiments. Chemical shifts are given in δ ppm based on the solvent used (77.1 ppm for $CDCl_3$ and 128.0 ppm for C_6D_6). [b] ^{1}H NMR spectra were measured at 500 MHz in $CDCl_3$ and C_6D_6 solutions, respectively. Chemical shifts are given in δ ppm based on $CHCl_3$ (7.26 ppm) in $CDCl_3$ and C_6H_6 (7.27 ppm) in C_6D_6, respectively. J values in parentheses are expressed in hertz.

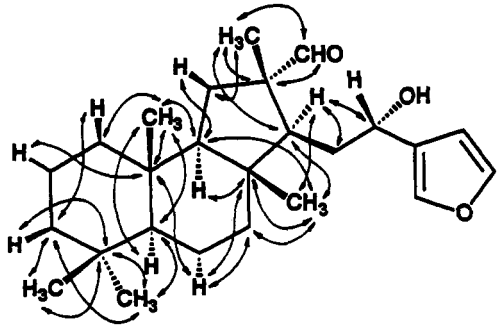


Figure 1. ^{13}C–^{1}H long-range correlations (in C_6D_6).

Formyl and secondary hydroxy groups were confirmed present by the following chemical reactions. The acetylation of 1 with acetic anhydride gave acetate 2 [δ_H 2.03 (3 H, s), 5.64 (1 H, m)]. The sodium chlorite oxidation[7] of 1 gave acid 3, which subsequently underwent conversion to methyl ester 4 by treatment with diazomethane. The PCC oxidation of 4 gave keto ester 5, whose ketonic carbonyl was noted to be conjugated with the furan moiety: ^{1}H NMR signals of the furan shifted downfield on proceeding from 1 to 5 [δ 6.76 (dd, J = 0.7, 1.6 Hz), 7.42 (t, J = 1.6 Hz), 8.07 (br s)]. The presence of a $CHCH_2CO$ group in 5 was indicated by the ^{1}H NMR spectrum [δ 2.50 (dd, J = 6.7, 8.3 Hz), 2.65 (dd, J = 6.7, 15.3 Hz), 2.81 (dd, J = 8.3, 15.3 Hz)], which showed a 2-(3-furanyl)-2-hydroxyethyl group to be present in 1. The positions of the formyl group at C-13 and 2-(3-furanyl)-2-hydroxyethyl group at C-14 were determined by two-dimensional ^{13}C–^{1}H long-range correlations (COLOC in C_6D_6, two or three bonds) as shown in Figure 1. Correlations of H-12/C-13, H-24/C-12, and H-24/C-13 indicated the formyl and methyl (C-24) groups to be situated at C-13, while those of H-14/C-15, H-14/C-16, and H-24/C-14 indicated the 2-(3-furanyl)-2-hydroxyethyl group to be bonded to the C-14 methine carbon.

Connection of the partial structures of 1 was made based on two-dimensional ^{13}C–^{1}H long-range correlations as

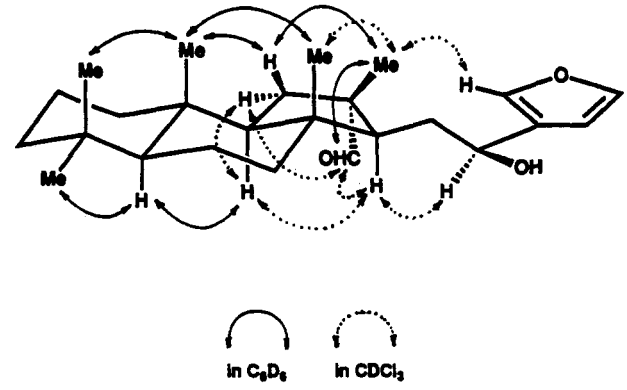
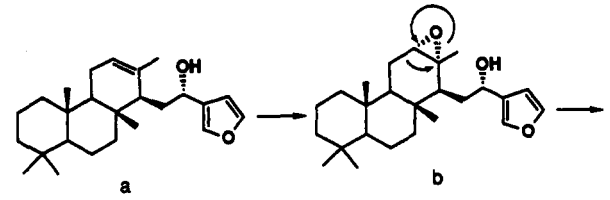
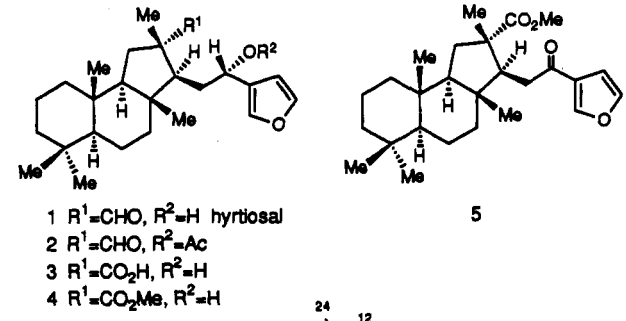
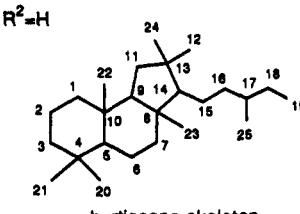


Figure 2. NOE correlations.

Scheme I. Possible Biosynthesis for Hirtiosal







hyrtiosane skeleton

shown in Figure 1, to give a tricarbocyclic plane structure involving a 4,4,8,10-tetramethyldecalin system for hyrtiosal (1). Correlations of H-1/C-3, H-3/C-4, H-20/C-4, H-21/C-4, and H-20/C-5 indicated the presence of a CH_2C-$H_2CH_2C(CH_3)_2CHCH_2CH_2$ group from C-1 to C-7. This finding along with correlations of H-23/C-7, H-23/C-8, H-23/C-9, H-9/C-8, H-22/C-9, H-22/C-10, H-22/C-1, H-22/C-5, and H-5/C-22 demonstrated the presence of a 4,4,8,10-tetramethyldecalin system. Correlations of H-11/C-9, H-24/C-11, and H-23/C-14 showed a cyclopentane ring to have condensed with the 4,4,8,10-tetramethyldecalin at C-8 and -9 positions.

The stereostructure of 1 was elucidated from two-dimensional NOESY spectra. The relative stereochemistries of chiral centers at positions C-8, -9, -13, and -14 in the B and C rings were determined from NOE correlations measured in $CDCl_3$ solution, as shown in Figure 2 by dashed-lined arrows. Such NOE correlations between methyl signals attached to A, B ring chiral centers could not be clearly determined, owing to similar chemical shifts of the methyl signals. The two-dimensional NOESY spectrum was thus measured in C_6D_6 solution in which the corresponding methyl signals were separated from each other. The NOE correlations shown in Figure 2 as solid-lined arrows indicate a trans-anti-trans configuration for the ring junction of A/B and B/C rings. The relative stereochemistry of the chiral center at C-16 bearing a hydroxy group on the side chain was also suggested by NOE correlations (in $CDCl_3$) between H-14 and H-16, and H-24 (CH_3) and H-25 (furan). These NOE correlations could be explained only in the case of configurations of 13*S**, 14*S**, and 16*S** using a molecular model. These relative stereochemistries are rationalized by the fact that several tetracarbocyclic sesterterpenoids from the sponge, *Hyrtios erecta*,[5] each has an oxygen function at C-16 and all have the relative configurations of 14*S** and 16*S** that are the same as those of 1.

Hyrtiosal (1) has a previously unknown carbon skeleton: we propose the name "hyrtiosane" for this new skeleton. Although numerous sponge-derived sesterterpenoids[2b,8] have been found, only a few sesterterpenoids possessing a tricarbocyclic skeleton have been reported.[9,10] A comparison of the structure of hyrtiosal with that of luffolide[9] with a cheilanthane skeleton[11] clearly indicated what would be a possible biosynthesis of the hyrtiosane skeleton. As shown in Scheme I, the olefinic bond of the tricarbocyclic precursor a containing a cheilanthane skeleton is oxidized to give the epoxy intermediate b, the subsequent rearrangement of which produces a ring-contracted hyrtiosane skeleton.

Experimental Section

Melting points are uncorrected. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ and C_6D_6 solutions. The conditions for NMR measurements are indicated in the footnotes of Table I. Two-dimensional NMR (500 MHz for ^1H and 125 MHz for ^{13}C) spectra were also measured in $CDCl_3$ and C_6D_6 solutions. EIMS spectra were obtained at 70 eV.

Extraction and Isolation. Wet specimens[6] of *Hyrtios erectus* (2.2 kg), collected on the coral reef of Ishigaki Island (Okinawa, Japan) in November 1988 were extracted with MeOH. The MeOH extract was suspended in water and extracted with EtOAc. The EtOAc-soluble portion (3.7 g) was chromatographed on a silica gel column. Stepwise elution with hexane–EtOAc (5:1 and then 1:1), EtOAc, and MeOH gave five fractions. The first fraction (0.93 g) was further subjected to repeated silica gel column chromatography (hexane–EtOAc, 4:1) followed by purification by preparative TLC (hexane–EtOAc, 4:1, developed three times) to give hyrtiosal (1) (21 mg).

Hyrtiosal (1): $[\alpha]_D$ −73.8° (*c* 0.42, $CHCl_3$); EIMS *m/z* 386 (M^+); HREIMS M^+ *m/z* obsd 386.2815, $C_{25}H_{38}O_3$ required 386.2818; ^1H NMR ($CDCl_3$ and C_6D_6) see Table I; ^{13}C NMR ($CDCl_3$ and C_6D_6) see Table I.

Acetylation of Hyrtiosal (1). To a stirred solution of 1 (47.5 mg) in pyridine (0.5 mL) was added acetic anhydride (0.5 mL). After being stirred at room temperature overnight, the mixture was diluted with EtOAc. The mixture was washed successively with H_2O, saturated $NaHCO_3$ solution, H_2O, saturated $CuSO_4$ solution, and H_2O. The organic layer was dried over anhyd Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane–EtOAc, 15:1) to give acetate **2** (30 mg): colorless rods; mp 141–2 °C; $[\alpha]_D$ −58.2° (*c* 0.71, $CHCl_3$); EIMS *m/z* 428 (M^+); HREIMS M^+ − AcOH *m/z* obsd 368.2710, $C_{25}H_{36}O_2$ required 368.2712; IR (KBr) 1719, 1250 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (3 H, s), 0.85 (3 H, s), 0.86 (3 H, s), 0.90 (3 H, s), 1.14 (3 H, s), 2.03 (3 H, s), 5.64 (1 H, m), 6.35 (1 H, br s), 7.37 (1 H, t, *J* = 1.6 Hz), 7.40 (1 H, br s), 9.35 (1 H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.8 (CH_3), 16.5 (CH_3), 18.3 (CH_2), 19.0 (CH_2), 19.3 (CH_3), 21.3 (2 CH_3), 30.8 (CH_2), 33.1 (C), 33.5 (CH_3), 33.6 (CH_2), 36.9 (C), 40.2 (CH_2), 40.8 (CH_2), 42.5 (CH_2), 44.8 (C), 48.9 (CH), 52.4 (C), 57.3 (CH), 60.2 (CH), 66.8 (CH), 108.8 (CH), 124.7 (C), 140.8 (CH), 143.3 (CH), 170.3 (C), 203.8 (CH).

Oxidation of Hyrtiosal (1). To a stirred solution of 1 (3.0 mg) in a mixture of *t*-BuOH and 2-methyl-2-butene (4:1, 0.3 mL) was added a solution (0.1 mL) of $NaClO_2$ (5.3 mg) and NaH_2PO_4 (5.3 mg). After being stirred for 90 min at 10 °C, the mixture was diluted with EtOAc, washed with H_2O, dried over anhyd Na_2SO_4, and concentrated under reduced pressure. The residue was treated with excess CH_2N_2 in diethyl ether at 0 °C. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (hexane–EtOAc, 5:1) to give methyl ester **3** (3.2 mg): colorless crystals; EIMS *m/z* 416 (M^+); HREIMS M^+ *m/z* obsd 416.2912, $C_{26}H_{40}O_4$ required 416.2924; ^1H NMR (400 MHz, $CDCl_3$) δ 0.81 (3 H, s), 0.82 (3 H, s), 0.84 (6 H, s), 1.29 (3 H, s), 2.10 (1 H, dd, *J* = 3.7, 10.7 Hz), 2.15 (1 H, dd, *J* = 6.1, 12.7 Hz), 3.70 (3 H, s), 4.60 (1 H, dd, *J* = 3.8, 9.5 Hz), 6.40 (1 H, br s), 7.37 (1 H, t, *J* = 1.7 Hz), 7.39 (1 H, br s).

Oxidation of Methyl Ester 3. To a stirred solution of **3** (2.2 mg) in CH_2Cl_2 (0.7 mL) were added powdered 4A molecular sieves (4 mg) and PCC (4 mg). After the mixture was stirred for 30 min at room temperature, additional molecular sieves (4 mg) and PCC (4 mg) were added, and the resultant mixture was further stirred for 50 min at room temperature. The mixture was diluted with diethyl ether and passed through a silica gel short column. The eluate was concentrated under reduced pressure, and the residue was purified by preparative TLC (hexane–EtOAc, 5:1) to give keto ester **4** (0.5 mg): colorless solids; EIMS *m/z* 414 (M^+); HREIMS M^+ *m/z* obsd 414.2766, $C_{26}H_{38}O_4$ required 414.2767; ^1H NMR (400 MHz, $CDCl_3$) δ 0.82 (3 H, s), 0.83 (3 H, s), 0.85 (3 H, s), 0.89 (3 H, s), 1.21 (3 H, s), 2.50 (1 H, dd, *J* = 6.7, 8.3 Hz), 2.65 (1 H, dd, *J* = 6.7, 15.3 Hz), 2.81 (1 H, dd, *J* = 8.3, 15.3 Hz), 3.69 (3 H, s), 6.76 (1 H, dd, *J* = 0.7, 1.6 Hz), 7.42 (1 H, t, *J* = 1.6 Hz), 8.07 (1 H, br s).

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Supplementary Material Available: ^1H NMR spectra of 1, 2, 3, and 4 and the ^{13}C NMR spectra of 1 and 2 (7 pages). Ordering information is given on any current masthead page.

(8) Recent example: (a) Ortiz, M.; Rodriguez, A. D. *Tetrahedron* **1990**, *46*, 1119. (b) Bheemasankara Rao, Ch.; Kalidindi, S. H. S. N.; Trimurtulu, G.; Venkata Rao, D. *J. Nat. Prod.* **1991**, *54*, 364. (c) Zeng, L.; Fu, X.; Su, J.; Pordesimo, E. O.; Traeger, S. C.; Schmitz, F. J. *J. Nat. Prod.* **1991**, *54*, 421.

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