# New Cytotoxic Furanosesterterpenes from an Okinawan Marine Sponge, *Ircinia* sp.

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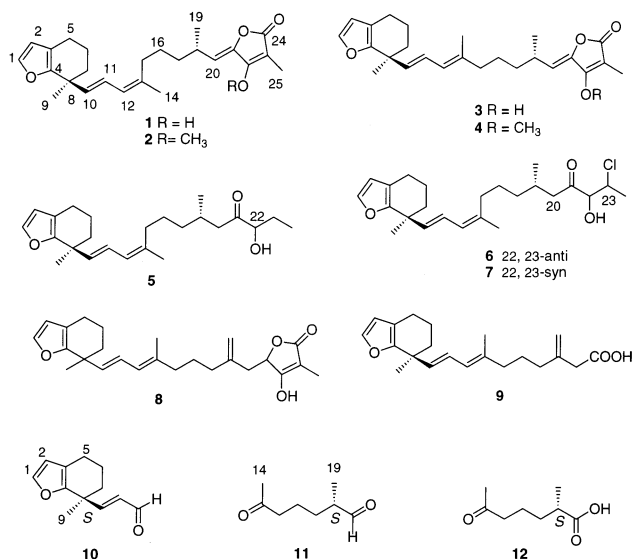
*Received September 25, 2002*

Five new sesterterpenes have been isolated from a lipophilic extract of a sponge, *Ircinia* sp., and their structures elucidated by spectroscopic and chemical methods. The absolute configurations of two metabolites (**1**, **3**) were established by chemical degradation. These compounds showed moderate cytotoxicity against KB cells.

In the course of our research on bioactive compounds from coral reef organisms, we found that the EtOAc extract from a sponge of the genus *Ircinia* (family Irciniidae) showed moderate cytotoxicity. Chromatographic separation of this extract led to the isolation of five new terpenoids (**1**, **3**, **5**–**7**) having a tetrahydrobenzofuran as a common moiety. Compounds **1** and **3** are sesterterpenes containing a tetronic acid, while **5**–**7** are related norsesterterpenes. These compounds are closely related to the known sponge metabolites hippospongin (**8**)[1] and untenic acid (**9**).[2] In this paper we report the isolation, structure elucidation, and biological activities of these new metabolites.




## Results and Discussion

A frozen sample of the sponge was extracted with acetone. The extract was concentrated, and the residue was partitioned between EtOAc and $H_2O$. The organic layer was chromatographed on silica gel followed by reversed-phase HPLC to yield compounds **1**, **3**, and **5**–**7**.

Compound **1** was a labile, colorless oil, $[\alpha]^{25}_{D}$ +42° ($CHCl_3$), having a molecular formula $C_{25}H_{32}O_4$ as determined by HREIMS. The $^1H$ NMR spectrum revealed the presence of $\alpha$ and $\beta$ protons [$\delta$ 7.25 (d, $J$ = 1.8 Hz), 6.25 (m)] of a 4,5-disubstituted furan, three olefinic protons [$\delta$ 5.57 (dd, $J$ = 15.3, 3 Hz), 5.90 (dd, $J$ = 11.0, 15.3 Hz), 5.80

**Table 1.** NMR Data ($CDCl_3$) for Compounds **1** and **3**

| | **1** | | | **3** | | |
|---|---|---|---|---|---|---|
| C no. | $\delta_C$ | | $\delta_H$ (mult., $J$ = Hz) | $\delta_C$ | | $\delta_H$ (mult., $J$ = Hz) |
| 1 | 140.6 | CH | 7.25 (d, 1.8) | 140.6 | CH | 7.25 (d, 1.8) |
| 2 | 110.2 | CH | 6.25 (m) | 110.2 | CH | 6.20 (s) |
| 3 | 116.6 | C | | 116.7 | C | |
| 4 | 154.4 | C | | 154.5 | C | |
| 5 | 22.5 | $CH_2$ | 2.42 (m) | 22.5 | $CH_2$ | 2.42 (m) |
| 6 | 20.0 | $CH_2$ | 1.72 (m) | 20.1 | $CH_2$ | 1.71 (m) |
| 7 | 38.3 | $CH_2$ | 1.77 (m)<br>1.60 (m) | 38.4 | $CH_2$ | 1.80 (m)<br>1.62 (m) |
| 8 | 38.6 | C | | 38.6 | C | |
| 9 | 25.8 | $CH_3$ | 1.35 (s) | 25.8 | $CH_3$ | 1.35 (s) |
| 10 | 138.5 | CH | 5.57 (dd, 15.3, 3) | 138.7 | CH | 5.60 (d, 15.3) |
| 11 | 125.0 | CH | 5.90 (dd, 15.3, 11) | 125.0 | CH | 5.90 (dd, 11, 15.3) |
| 12 | 125.5 | CH | 5.80 (brd, 11) | 124.6 | CH | 5.78 (d, 11) |
| 13 | 137.6 | C | | 137.3 | C | |
| 14 | 23.7 | $CH_3$ | 1.70 (s) | 16.5 | $CH_3$ | 1.62 (s) |
| 15 | 32.1 | $CH_2$ | 2.00 (m) | 39.8 | $CH_2$ | 2.00 (m) |
| 16 | 25.8 | $CH_2$ | 1.35 (m) | 25.6 | $CH_2$ | 1.35 (m) |
| 17 | 36.7 | $CH_2$ | 1.34 (m) | 36.8 | $CH_2$ | 1.31 (m) |
| 18 | 30.9 | CH | 2.80 (brs) | 30.7 | CH | 2.79 (brs) |
| 19 | 20.6 | $CH_3$ | 1.05 (d, 6.7) | 20.6 | $CH_3$ | 1.05 (d, 6.7) |
| 20 | 115.6 | CH | 5.26 (d, 10) | 115.6 | CH | 5.25 (d, 10) |
| 21 | 142.6 | C | | 142.9 | C | |
| 22 | 162.1 | C | | 162.3 | C | |
| 23 | 99.3 | C | | 99.5 | C | |
| 24 | 171.8 | C | | 171.7 | C | |
| 25 | 6.1 | $CH_3$ | 1.80 (s) | 6.1 | $CH_3$ | 1.80 (s) |

(brd, $J$ = 11 Hz)] of a conjugated diene, and four methyl groups (Table 1). These signals, together with UV absorption at $\lambda_{max}$ 240 nm and IR bands ($\nu_{max}$ 1731, 1643 $cm^{-1}$), suggested that **1** was a sesterterpene tetronic acid related to **8** and variabilin.[3] HMBC correlations from H-5 ($\delta$ 2.24 m) and H-9 ($\delta$ 1.35 s) to C-4 ($\delta$ 154.4 s) and from H-7 ($\delta$ 1.75 m, 1.60 m) to C-5 ($\delta$ 22.5 t) revealed the presence of a tetrahydrobenzofuran skeleton. The presence of the ring portion was further supported by an EIMS fragment ion at $m/z$ 135 ($C_9H_{11}O$) formed by cleavage of the bond between C-8 and C-10. Irradiation of the signals for H-9 and H-7 caused enhancement of the H-11 and H-10 resonances, respectively, suggesting the proximity of the ring to a conjugated double bond. Comparison of the $^1H$ NMR data with those of hippospongin (**8**), having the same molecular formula and the tetrahydrobenzofuran moiety, showed that these compounds were very similar. The most notable difference was the presence of three proton resonances ($\delta$ 4.7–4.9) due to two exomethylene and one oxymethine proton in the spectrum of **8**, which are absent

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10.1021/np020456p CCC: $25.00 © 2003 American Chemical Society and American Society of Pharmacognosy
Published on Web 01/24/2003

in **1**. Instead, **1** showed a signal at δ 5.26 (d, $J$ = 10 Hz) and no signal corresponding to that of an oxymethine proton. This observation suggested the presence of a double bond between C-20 and C-21 instead of the C-18/C-19 unsaturation in **8**.

The sequence of $C_1-C_{19}$ was unambiguously assigned by analysis of the HMQC, HMBC, and $^1H-^1H$ COSY spectra and also by comparison of the NMR data with those of hippospongin. The connection between this portion and the tetronic acid moiety was inferred from HMBC correlation between H-19 (δ 1.05 d) and C-20 (δ 116.8 d) and COSY correlation observed between H-18 (δ 2.80 brm) and H-20 (δ 5.26 d), establishing the planar structure of **1**. The $E$ geometry of the C-10/C-11 double bond was deduced from the coupling constant ($J$ = 15.3 Hz) of the signals assigned for H-10 (δ 5.57 dd) and H-11 (δ 5.90 dd). The geometry of the C-12/C-13 double bond was shown to be $Z$ by the chemical shift (δ 23.7) of the methyl group at C-13.[4] It was also confirmed by NOE observation between the resonances of H-12 and H-14. The double bond geometry at C-20 was indicated by a difference NOE experiment on the methylation product **2**. Irradiation of the H-20 signal (δ 5.26) caused enhancement of the methoxyl signal at δ 4.12, suggesting Z geometry of the double bond.

The absolute configuration of **1** was determined by degradation. Oxidation of **1** with $RuO_2-NaIO_4$ gave two products, a new aldehyde (**10**) and the known 2-methyl-6-oxoheptanal (**11**).[5] The aldehyde **10** was analyzed for $C_{12}H_{14}O_2$ by HREIMS. The $^1H$ NMR spectrum of **10** contained signals for an aldehyde proton at δ 9.54 (d, $J$ = 7.6 Hz), two furan protons (δ 7.28, 6.21), as in **1**, and a pair of olefinic protons [δ 6.84 (d, $J$ = 15.6 Hz), 5.85 (dd, $J$ = 15.6, 7.6 Hz)]. These data were consistent with the structure shown as **10**. The optical rotation of **10**, $[\alpha]_D$ +18.5°, suggested that it has an 8$S$ configuration by application of the Brewster rule,[6] as in the case of untenic acid (**9**).[2] The keto-aldehyde **11** showed an optical rotation ($[\alpha]_D$ +7.2°) corresponding to the $S$-enantiomer.[5] Prolonged oxidation of **1** with an excess amount of the reagents gave (+)-($S$)-2-methyl-6-oxoheptanoic acid (**12**), an enantiomer of the known acid.[5] Both **11** and **12** were identified by comparison of the spectroscopic data with those reported.[5] Thus, the absolute configurations of **1** were elucidated as 8$S$ and 18$S$.

Compound **3** was isolated as an unstable yellow oil with a molecular formula $C_{25}H_{32}O_4$. $^1H$ and $^{13}C$ NMR analysis (Table 1) of **3** revealed considerable similarity to **1**, with one significant difference observed for a methyl signal at δ 16.5 (C-14), indicating 12$E$ geometry. This was further substantiated by observing an NOE between H-11 (δ 5.90 dd) and H-14 (δ 1.62 s). The same 20$Z$ double bond geometry was shown by NOE measurement on the methyl derivative **4**. The cofigurations at the chiral centers were similarly determined by degradation of **3**, yielding the same products **10** and **11**. Thus, **3** was shown to be a geometric isomer of **1**.

Compound **5** was a pale yellow oil displaying a molecular ion at $m/z$ 372, high-resolution measurement of which established the molecular formula $C_{24}H_{36}O_3$ (Δ +1.5 mmu). The partial structure for the C-1/C-19 portion was assigned by NMR data (Tables 2 and 3) as in **1**. The presence of a ketone and a secondary alcohol was indicated by the signals at δ 211.2 s and 83.6 d and also δ 4.66 (dd, $J$ = 7, 5 Hz). The IR spectrum also revealed the presence of these groups (3465, 1716 $cm^{-1}$). The triplet methyl signal at δ 0.93 (t, $J$ = 7.3 Hz) showed the presence of a terminal ethyl group. Connectivity of these functional groups was made by 2D NMR data to construct the remaining C-20/C-24 portion, thus establishing the structure of **5**. We assume the same configurations at C-8 and C-18 as those of **1** and **3**. The configuration at C-22 remains to be solved.

Compound **6** was an unstable, pale yellow oil, with a molecular formula $C_{24}H_{35}ClO_3$ (Δ −0.1 mmu). The same partial structure for C-1/C-19 was confirmed by NMR data (Tables 2 and 3) as in **5**. The remaining portion, $C_5H_8ClO_2$, contained a ketone (δ 207.4) and a chlorohydrin moiety as elucidated by 2D NMR analysis and IR absorption bands (3477, 1722 $cm^{-1}$). The COSY spectrum indicated a strong correlation between two methine proton signals at δ 4.99 (H-22) and 4.52 (H-23) and the latter with methyl proton resonance at δ 1.62 (d, H-24). The 2D NMR analysis allowed us to connect the C-21/C-24 moiety to C-18 through C-20 methylene ($\delta_H$ 2.50, 2.60; $\delta_C$ 46.6).

Compound **7** was shown to have a molecular formula, $C_{24}H_{35}ClO_3$, identical to **6** by HREIMS (Δ −0.5 mmu). The spectral features of **7** were very similar to those of **6**, with notable differences being found in the $^1H$ NMR signals at

**Table 2.** $^{13}C$ NMR Data for **5**−**7** in $CDCl_3$[a]

| C no. | 5 | | 6 | | 7 | |
|---|---|---|---|---|---|---|
| 1 | 140.7 | CH | 140.7 | CH | 140.7 | CH |
| 2 | 110.1 | CH | 110.2 | CH | 110.2 | CH |
| 3 | 116.6 | C | 116.7 | C | 116.6 | C |
| 4 | 154.8 | C | 154.4 | C | 154.6 | C |
| 5 | 22.5 | $CH_2$ | 22.6 | $CH_2$ | 22.5 | $CH_2$ |
| 6 | 20.1 | $CH_2$ | 20.1 | $CH_2$ | 20.1 | $CH_2$ |
| 7 | 38.3 | $CH_2$ | 38.4 | $CH_2$ | 38.3 | $CH_2$ |
| 8 | 38.6 | C | 38.6 | C | 38.6 | C |
| 9 | 25.8 | $CH_3$ | 25.8 | $CH_3$ | 25.8 | $CH_3$ |
| 10 | 138.5 | CH | 138.5 | CH | 138.5 | CH |
| 11 | 124.8 | CH | 124.8 | CH | 124.8 | CH |
| 12 | 125.4 | CH | 125.4 | CH | 125.5 | CH |
| 13 | 137.6 | C | 137.6 | C | 137.6 | C |
| 14 | 23.7 | $CH_3$ | 23.8 | $CH_3$ | 23.7 | $CH_3$ |
| 15 | 32.3 | $CH_2$ | 32.3 | $CH_2$ | 32.3 | $CH_2$ |
| 16 | 25.4 | $CH_2$ | 25.4 | $CH_2$ | 25.4 | $CH_2$ |
| 17 | 36.8 | $CH_2$ | 36.7 | $CH_2$ | 36.5 | $CH_2$ |
| 18 | 28.3 | CH | 28.1 | CH | 27.9 | CH |
| 19 | 19.6 | $CH_3$ | 19.6 | $CH_3$ | 19.5 | $CH_3$ |
| 20 | 45.9 | $CH_2$ | 46.6 | $CH_2$ | 47.7 | $CH_2$ |
| 21 | 211.2 | C | 207.4 | C | 207.9 | C |
| 22 | 83.6 | CH | 84.2 | CH | 84.1 | CH |
| 23 | 24.6 | $CH_2$ | 56.2 | CH | 55.3 | CH |
| 24 | 9.0 | $CH_3$ | 21.6 | $CH_3$ | 20.9 | $CH_3$ |

[a] Measured at 125 MHz. Assignments were aided by DEPT, HMQC, and HMBC experiments.

**Table 3.** $^1H$ NMR Data (500 MHz) for **5**−**7** in $CDCl_3$[a]

| position | 5 | 6 | 7 |
|---|---|---|---|
| 1 | 7.24 (s) | 7.25 (s) | 7.25 (m) |
| 2 | 6.15 (d, 1.8) | 6.15 (d, 1.8) | 6.15 (d, 1.8) |
| 5 | 2.40(m) | 2.39 (m) | 2.39 (m) |
| 6 | 1.70 (m) | 1.70 (m) | 1.70 (m) |
| 7 | 1.62 (m), 1.75 (m) | 1.62 (m), 1.75 (m) | 1.62 (m), 1.75 (m) |
| 9 | 1.34 (s) | 1.34 (s) | 1.34 (s) |
| 10 | 5.57 (d, 15.3) | 5.57 (d, 15.3) | 5.57 (d, 15.3) |
| 11 | 5.91 (dd, 15.3, 11) | 5.91 (dd, 15.3, 11) | 5.91 (dd, 15.3, 11) |
| 12 | 5.76 (d, 11) | 5.77 (d, 11) | 5.77 (d, 11) |
| 14 | 1.70 (s) | 1.70 (s) | 1.70 (s) |
| 15 | 2.00 (m) | 2.00 (m) | 2.00 (m) |
| 16 | 1.34 (m) | 1.34 (m) | 1.34 (m) |
| 17 | 1.10 (m), 1.24 (m) | 1.10 (m), 1.24 (m) | 1.10 (m), 1.24 (m) |
| 18 | 2.00 (m) | 2.00 (m) | 2.00 (m) |
| 19 | 0.83 (d, 6.7) | 0.87 (d, 6.7) | 0.88 (dd, 6, 1.5) |
| 20 | 2.51 (m) | 2.50 (dd, 18, 8) | 2.58 (dd, 18, 8) |
| | 2.40 (m) | 2.60 (m) | 2.66 (m) |
| 22 | 4.66 (dd, 7, 5) | 4.99 (br s) | 4.85 (d, 5.5) |
| 23 | 1.85 (m), 1.72 (m) | 4.52 (m) | 4.36 (m) |
| 24 | 0.93 (t, 7.3) | 1.62 (d, 6.7) | 1.56 (d, 6.7) |

[a] Signal multiplicity and coupling constants (Hz) are shown in parentheses.

$\delta$ 4.71 (d, C*H*OH) and 4.2 (m, C*H*Cl). This suggested that **7** was isomeric to **6** at the chlorohydrin functionality. HMBC and COSY analysis for the C-20/C-24 portion revealed the same arrangements for the ketone ($\delta$ 207.9), hydroxyl, and chlorine atom with those of **6,** and this portion was connected to C-18. Therefore, **6** and **7** must be diastereomers. It has been demonstrated[7] in the enantiomeric analysis of halohydrins that a *syn* diastereomer exhibits proton signals for the C*H*-OH ($\delta$ 3.88) and C*H*-Cl ($\delta$ 3.48−3.55) at higher fields than corresponding signals ($\delta$ 3.94 and 3.60, respectively) of the *anti* isomer. Thus, **7** is suggested to have a *syn* cholorohydrin moiety, while **6** has an *anti* isomeric structure.

All compounds isolated were evaluated for cytotoxicity against KB cells. Compounds **1** and **3** showed an $IC_{50} \geq 1$ $\mu$g/mL, while **5**−**7** exhibited an $IC_{50} < 1$ $\mu$g/mL.

## Experimental Section

**General Experimental Procedures.** The optical rotations were recorded with a Jasco DIP-1000 digital polarimeter. UV spectra were taken using a Jasco UVDEC 610 spectrometer. IR spectra were measured on a Jasco FT IR-300 spectrometer. NMR spectra were taken on a JEOL $\alpha$ 500 FT NMR spectrometer in $CDCl_3$ and referenced to the $CHCl_3$ solvent signal at $\delta$ 7.26 for $^1H$ NMR and $\delta$ 77.0 for $^{13}C$ NMR spectra. Multiplicities of $^{13}C$ spectra were assigned by DEPT experiments. EIMS mass spectra were measured on a Hitachi M-2500 instrument. HPLC separations were carried out on a Hitachi L-6000 or Shimadzu LC 9A pumps equipped with a Waters 486 or Hitachi L-4000 UV detector and Waters R401 differential refractometer. Columns used for HPLC were normal-phase silica (250 × 10 mm, 5 $\mu$m, LiChrosorb) and reversed-phase Nacalai 5C18-AR II (10 × 250 mm). Kieselgel 60 (230−400 mesh) silica gel was used for column chromatography. TLC was carried out on precoated silica 60 F254 plates and visualized with vanillin−EtOH−1% $H_2SO_4$.

**Animal Material.** A sponge sample, *Ircinia* sp. (Demospongiae, Dictyoceratida, Irciniidae), was collected by hand using scuba in Iriomote Island, Okinawa, in May 1998. Taxonomic examination of the sponge was made by Dr. John N. A. Hooper, Queensland Museum, South Brisbane, Queensland, Australia. The sponge forms massive, subspherical, with large pointed conules covering the entire surface and groups of oscules near the summits of each sponge. The color is gray exterior, orange to beige interior, no color change in EtOH. The pores are small, scattered over the surface between conules. The texture is tough, difficult to tear, and compressible. The ectosomal skeleton is without a cortical armor but with a delicate tracery of sand running along ridges between adjoining conules. The choanosomal skeleton consists of few primary fibers cored by detritus and very few or no secondary or tertiary fibers interconnecting. The collagen filaments are abundant in whorls of wool, almost totally occupying the mesohyl. The filaments are rarely knobbed. This is relatively common throughout the outer Great Barrier Reef. Its record in Japan indicates that it is probably a common member of the wider Indo-West Pacific fauna. The sponge cannot presently be assigned to a known species. A voucher specimen (OK-98-61) is kept at the Department of Chemistry, Biology, and Marine Science, University of the Ryukyus.

**Extraction and Isolation.** A sample of frozen sponge (1.0 kg wet weight) was cut into small pieces (2 cm) and soaked in acetone (1L) for 14 h. After decantation, fresh solvent was added, and the procedure was repeated three times. The combined extracts were concentrated and partitioned between EtOAc and $H_2O$. The organic layer was concentrated to give an oil (6.55 g). The oil was chromatographed on silica gel by eluting with a step gradient of hexane−$CH_2Cl_2$−EtOAc−MeOH. Eight fractions were obtained. On the basis of the characteristic signals observed in $^1H$ NMR spectra, fractions II and V were selected for further purification using HPLC (RP-18, MeOH−$H_2O$) to afford compounds **1** and **3** from fraction II and compounds **5**−**7** from fraction V.

**Compound 1:** pale yellow oil (85.1 mg); $[\alpha]^{30}_D$ +42° (*c* 0.34, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 240 (4.4) and 310 nm (3.4); IR (film) $\nu_{max}$ 3450, 2930, 1731, 1643, and 1435 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 1; EIMS *m*/*z* 397 [$(M + 1)^+$, 98], 382 (100), 324 (6), 228 (5), 202 (50), 174 (25), 148 (36), 121 (58), 91 (16); HREIMS *m*/*z* 396.2299 [$M^+$] (calcd for $C_{25}H_{32}O_4$, 396.2299).

**Compound 3:** pale yellow oil (15.1 mg); $[\alpha]^{30}_D$ +30.2° (*c* 0.34, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 240 (4.4) and 310 nm (3.2); IR (film) $\nu_{max}$ 3446, 2931, 2868, 1739, 1643, and 1455 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Table 1; HREIMS *m*/*z* 396.2283 [$M^+$] (calcd for $C_{25}H_{32}O_4$, 396.2299).

**Compound 5:** pale yellow oil (3.0 mg); $[\alpha]^{30}_D$ +12.2° (*c* 0.2, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 242 nm (4.4); IR (film) $\nu_{max}$ 3465, 1716, 1637, 1455, 1378, 1263, and 740 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 2 and 3; EIMS *m*/*z* 372 ($M^+$, 100), 357 (92), 339 (10), 215 (7), 201 (55), 187 (9), 173 (22), 148 (34), 135 (80), 121 (60), 91 (18); HREIMS *m*/*z* 372.2677 [$M^+$] (calcd for $C_{24}H_{36}O_3$, 372.2662).

**Compound 6:** pale yellow oil (3.6 mg); $[\alpha]^{30}_D$ +3.3° (*c* 0.3, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 242 nm (4.3); IR (film) $\nu_{max}$ 3477, 1722, 1643, 1454, 1378, 1261, 1060, 1012, 978, 831, 737, and 652 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 2 and 3; EIMS *m*/*z* 406/408 ($M^+$, 57/20), 391/393 (65/28), 370 (90), 355 (100), 313 (10), 215 (8), 201 (75), 187 (15), 173 (35), 148 (55), 135 (86), 121 (85), 91 (22); HREIMS *m*/*z* 408.2271 (calcd for $C_{24}H_{35}{}^{35}ClO_3$, 406.2272).

**Compound 7:** pale yellow oil (12.1 mg); $[\alpha]^{30}_D$ +2.5° (*c* 0.85, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 242 nm (4.3); IR (film) $\nu_{max}$ 3477, 1716, 1645, 1454, 1377, 1234, 1014, and 831 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 2 and 3; EIMS *m*/*z* 406/408 ($M^+$, 74/25), 391/393 (88/27), 370 (85), 355 (100), 313 (10), 215 (8), 201 (88), 187 (15), 173 (40), 148 (65), 135 (92), 121 (98), 91 (37); HREIMS *m*/*z* 406.2267 (calcd for $C_{24}H_{35}{}^{35}ClO_3$, 406.2272).

**Methylation of 1.** To a solution of **1** (2.5 mg) in MeOH (0.5 mL) was added 10% trimethylsilyldiazomethane in hexane. The solution was allowed to stand at room temperature (15 min) and concentrated to dryness under a stream of nitrogen to yield a methyl derivative **2** (0.8 mg): $^1H$ NMR ($CDCl_3$) $\delta$ 7.26 (s, 1H), 6.18 (m, 1H), 5.92 (m, 1H), 5.81 (d, *J* = 10.3 Hz, 1H), 5.60 (dd, *J* = 15, 3.1 Hz, 1H), 5.15 (d, 10.3 Hz, 1H), 4.12 (s, $OCH_3$, 3H), 2.74 (m, 1H), 2.42 (m, 2H), 2.07 (s, 3H), 1.85 (m, 2H), 1.62 (s, 3H), 1.36 (s, 3H), 1.15 (m, 2H), 1.03 (d, *J* = 6.7 Hz, 3H).

**Methylation of 3.** Methyl derivative **4** (0.5 mg) was similarly prepared from **3** (2.0 mg) as above: $^1H$ NMR ($CDCl_3$) $\delta$ 7.27 (s, 1H), 6.18 (m, 1H), 5.96 (m, 1H), 5.78 (d, *J* = 10.3 Hz, 1H), 5.63 (dd, *J* = 15.3, 3.1 Hz, 1H), 5.14 (d, *J* = 10.3 Hz, 1H), 4.12 (s, $OCH_3$, 3H), 2.76 (m, 1H), 2.41 (m, 2H), 2.07 (s, 3H), 1.85 (m, 2H), 1.63 (s, 3H), 1.36 (s, 3H), 1.10 (m, 2H), 1.03 (d, *J* = 6.7 Hz, 3H).

**Oxidative Cleavage of 1.** To a mixture of **1** (40.0 mg) in acetonitrile (2 mL), sodium metaperiodate (0.2 mmol) in carbon tetrachloride (2 mL), and water (3 mL) was added ruthenium(IV) oxide (3 mg). After stirring at room temperature (3 h), the mixture was extracted with $CH_2Cl_2$ (20 mL), and the organic layer was concentrated. The crude product was separated using PTLC (Si 60, EtOAc−hexane 7:5) to give **10** (2.1 mg) and **11** **(**1.5 mg).

**Aldehyde 10:** $[\alpha]^{25}_D$ +18.5° (*c* 0.1, $CHCl_3$); IR ($CHCl_3$) $\nu_{max}$ 2920, 1714 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 9.54 (1H, d, *J* = 7.6 Hz), 7.28 (1H, d, *J* = 1.8 Hz), 6.21 (1H, d, *J* = 1.8 Hz), 6.84 (1H, d, *J* = 15.6 Hz), 5.85 (1H, dd, *J* = 15.6, 7.6 Hz), 2.44 (2H, m), 1.90 (1H, m), 1.60−1.70 (2H, m), 1.44 (3H, s); EIMS *m*/*z* 190 ($M^+$, 80), 175 (100), 161 (30), 147 (40), 135 (40), 119 (15), 108 (35), 91 (32); HREIMS *m*/*z* 190.1023 (calcd for $C_{12}H_{14}O_2$, 190.0994).

**Aldehyde 11:** colorless oil; $[\alpha]^{25}_D$ +7.2° (*c* 0.11, $CHCl_3$) [lit.[5] $[\alpha]^{25}_D$ +4.7° ($CHCl_3$)]; IR ($CHCl_3$) $\nu_{max}$ 2920, 1730 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 9.62 (1H, d, *J* = 2 Hz), 2.45 (3H, m), 2.12 (3H, s), 1.60−1.70 (4H, m), 1.20 (3H, d, *J* = 7 Hz). $^1H$ NMR data were identical with those reported for (+)-(*S*)-2-methyl-6-oxo heptanal.[5]

**Oxidative Cleavage of 3.** A solution of **3** (40.0 mg) in acetonitrile (2 mL) was treated in the same manner as above to give the aldehyde **10** (1.5 mg) and **11** (1.2 mg), which were identical in all respects with those obtained from **1**.

**Keto-acid 12.** Compound **1** (30 mg) was treated with an excess amount of $NaIO_4$ (5 mmol) and ruthenium(IV) oxide (3 mg) for an extended period (14 h) to yield keto-acid **12** (4 mg): colorless oil; $[\alpha]_D^{25}$ +8.2° (*c* 0.83, $CHCl_3$) [lit.[5] $[\alpha]^{25}_D$ −8.5° ($CHCl_3$)]; IR ($CHCl_3$) $\nu_{max}$ 3510, 2930, and 1714 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 2.45 (3H, m), 2.14 (3H, s), 1.63 (3H, m), 1.45 (1H, m), and 1.20 (3H, d, $J$ = 7 Hz). The $^1H$ NMR data were identical with those reported for (−)-(*R*)-2-methyl-6-oxo heptanoic acid.[5]

**Cytotoxicity Test.** The assay was conducted by M. Kobayashi and S. Aoki at Faculty of Pharmaceutical Sciences, Osaka University. KB cells were seeded in 100 $\mu$L of RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum and kanamycin (50 $\mu$g/mL). Cells were exposed to graded concentrations of sesterterpenes at 37 °C for 72 h in triplicate. Cytotoxicity was measured by using 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) calorimetric assay. Results were expressed as $IC_{50}$ determined by the concentration that reduced by 50% the optical density of treated cells with respect to the optical density of untreated controls.

**Acknowledgment.** We thank M. Kobayashi and S. Aoki, Osaka University, for the cytotoxicity assay. This work was partially supported by a Grant-in-Aid for Scientific Research (No. 10672109) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

## References and Notes

(1) Kobayashi, J.; Ohizumi, Y.; Nakamura, H.; Hirata, Y. *Tetrahedron Lett.* **1986**, *27*, 2113−2116.
(2) Shoji, N.; Umeyama, A.; Kishi, K.; Arihara, S.; Ohizumi, Y.; Kobayashi, J. *Aust. J. Chem.* **1992**, *45*, 793−795.
(3) Barrow, C. J.; Blunt, J. W.; Munro, M. H. G.; Perry, N. B. *J. Nat. Prod.* **1988**, *51*, 275−281.
(4) González, A. G.; Rodríguez, M. L.; Barrientos, A. S. M.*J. Nat. Prod.* **1983**, *46*, 256−261.
(5) Giersch, W.; Schulte-Elte, K. H. *Helv. Chim. Acta* **1990**, *73*, 733−738.
(6) Brewsters, J. H. *J. Am. Chem. Soc.* **1959**, *81*, 5493−5500.
(7) Besse, P.; Sokoltchik, T.; Veschambre, H. *Tetrahedron: Asymmetry* **1998**, *9*, 4441−4457.

NP020456P