bond via a cyclopropylmethyl anion type rearrangement or scission of the C2–C3 bond via a dianion intermediate. The *p*-tolylcyclopropane opens exclusively at C1–C2. The *o*-tolylcyclopropane gives products from both ring-opening pathways.

## Experimental Section

All organic reagents were obtained from Aldrich Chemical Co. The [1,3-bis(diphenylphosphino)propane]nickel(II) dichloride was obtained from Strem Chemicals. The *n*-butyllithium was obtained from FMC Corp. The potassium *tert*-butoxide was used without further purification. The hexane used in metalation reactions was washed with concentrated sulfuric acid and then distilled from calcium hydride. Tetrahydrofuran was distilled from sodium/benzophenone. NMR spectra were obtained on a 7.1-T, broadband QE300 NMR instrument operating at 300.2 MHz for proton, 75.4 MHz for $^{13}C$, and 46.1 MHz for deuterium. Chemical shifts are reported relative to TMS except in the case of the deuterium NMR, where the samples were run in chloroform and the shifts were obtained relative to $CD_2Cl_2$. Gas chromatographic analysis was carried out on a Hewlett-Packard chromatograph Model 5890A with a wide bore methylphenylsilicone capillary column, 30 m in length. Product yields were determined by both $^1H$ NMR and gas chromatography.

**Preparation of the Tolylcyclopropanes 2, 4, and 8.** Cyclopropylmagnesium bromide (0.18 mol in 125 mL of THF) was added via syringe to a stirred mixture of the bromotoluene (16.5 g, 0.097 mol), [1,3-bis(diphenylphosphino)propane]nickel(II) dichloride (0.25 g), and THF (125 mL) in a nitrogen-charged 250-mL round-bottom flask equipped with reflux condenser and septum. Upon completion of addition, the mixture was allowed to stir at room temperature for 2 h before the flask was placed in an oil bath and allowed to reflux for 72 h. The reaction was monitored by gas chromatography and shown to be complete by the disappearance of the bromotoluene. The reaction mixture was worked up by adding 100 mL of ether and washing with 5% HCl followed by several water washings. The organic layer was dried with magnesium sulfate. The solvent was removed using a rotary evaporator. The residue was vacuum distilled to give the tolylcyclopropane.

Compound **2** was isolated in 70% yield (bp 80–83 °C/18 Torr, lit.[11] bp 75–77 °C/12 Torr; 97% pure). $^1H$ NMR ($CDCl_3$): $\delta$ 0.68 (m, 2 H); 0.91 (m, 2 H); 1.86 (m, 2 H); 2.31 (s, 3 H); 6.87 (d, $J$ = 9.1 Hz, 1 H); 6.88 (s, 1 H); 6.95 (d, $J$ = 9.1 Hz, 1 H); 7.15 (t, $J$ = 9.1 Hz, 1 H).

Compound **4** was isolated in 70% yield (bp 80–83 °C/18 Torr, lit.[11] bp 80–81 °C/14 Torr; 99% pure). $^1H$ NMR ($CDCl_3$): $\delta$ 0.72 (m, 2 H); 0.98 (m, 2 H); 1.92 (m, 1 H); 2.37 (s, 3 H); 7.03 (d, $J$ = 7.8 Hz, 2 H); 7.12 (d, $J$ = 7.8 Hz, 2 H).

Compound **8** was isolated in 65% yield (80–83 °C/18 Torr, lit.[11] bp 70–71 °C/18 Torr; 98% pure). $^1H$ NMR ($CDCl_3$): $\delta$ 0.62 (m, 2 H); 0.90 (m, 2 H); 1.86 (m, 1 H); 2.41 (s, 3 H); 6.95 (m, 1 H); 7.04–7.14 (m, 3 H). NMR data for these compounds are consistent with that reported in the literature.[11]

**Preparation of 2-Cyclopropylpropene (13).** A 1-L three-necked flask charged with nitrogen was equipped with a magnetic stirrer, a pressure equalizing dropping funnel containing cyclopropylmagnesium bromide (385 mL, 1.3M in THF), a reflux condenser, 2-bromopropene (40.0 g, 0.33 mol), dichloro[1,3-bis-(diphenylphosphino)propane]nickel(II) (0.30 g), and anhydrous THF (185 mL). The Grignard reagent was added over 15–20 min to the ice-cooled mixture. The reaction mixture was then allowed to warm to room temperature. An exothermic reaction beings after about 30 min and the mixture starts to reflux. External heat was applied to allow the reflux to continue for 8 h. After removing the heat source and allowing the mixture to cool to room temperature, water was carefully added to discharge the excess Grignard reagent. Dilute HCl was added to help form a discrete organic layer. The organic layer was washed with water until it was nearly free of THF. The organic layer was then distilled (bp 73–77 °C; lit.[13] bp 70–80 °C) to give 2-cyclopropylpropene (17 g, 70%, 96% pure).

$^1H$ NMR ($CDCl_3$): $\delta$ 0.47 (m, 2 H); 0.59 (m, 2 H); 1.39 (m, 1 H); 1.62 (m, 3 H); 4.65 (m, 1 H); 4.69 (m, 1 H). Spectral data are consistent with literature values.[13]

**Metalation of the Tolylcyclopropanes.** In a typical metalation reaction, potassium *tert*-butoxide (2.50 g, 22.4 mmol), *n*-butyllithium (13.8 mL of 1.6 M *n*-butyllithium), the tolylcyclopropane (0.66 g, 5 mmol), and 20 mL of dry hexane were combined under nitrogen in a Schlenk flask equipped with a magnetic stirring bar. The heterogeneous reaction mixture was stirred at room temperature under nitrogen for at least 24 h before quenching or heating. If the reaction mixture was heated, the stopper was replaced by a reflux condenser and gas tube. The flask was placed in an oil bath, and the solvent was allowed to reflux with stirring while being kept under nitrogen.

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**Registry No.** **2**, 19714-73-9; **3**, 1124-20-5; **4**, 6921-43-3; **5**, 2077-30-7; **6**, 2077-29-4; **7**, 3333-13-9; **8**, 27546-46-9; **9**, 2077-34-1; **10**, 2077-33-0; **11**, 1587-04-8; **12**, 7399-49-7; **13**, 4663-22-3; **14**, 513-81-5; cyclopropylmagnesium bromide, 23719-80-4; 3-bromotoluene, 591-17-3; 4-bromotoluene, 106-38-7; 2-bromotoluene, 95-46-5; 2-bromopropene, 557-93-7.

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# Luffariolides A–E, New Cytotoxic Sesterterpenes from the Okinawan Marine Sponge *Luffariella* sp.

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Manoalide is an antimicrobial sesterterpene first isolated from the Palauan sponge *Luffariella variabilis*[2] that has proven to have analgesic and antiinflammatory activities[3] and inhibit the action of phospholipase $A_2$.[4] Recent studies have revealed that marine sponges of the genus *Luffariella* are a rich source of manoalide-related sesterterpenoids, and most of them possess useful bioactivities.[5] During our investigations of pharmacologically active substances from Okinawan marine organisms,[6] we recently examined extracts of the Okinawan sponge *Luffariella* sp.

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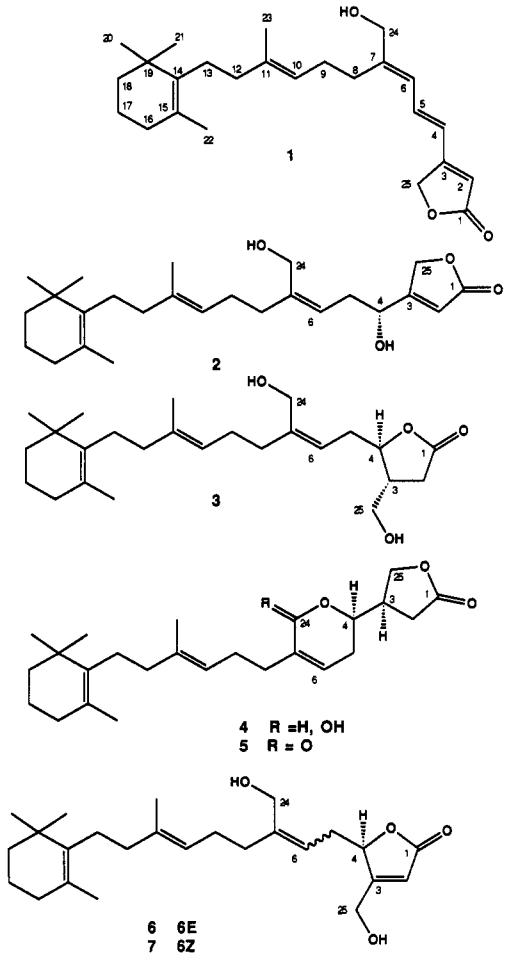
and isolated five new sesterterpenes, luffariolides A–E (1–5), with cytotoxic activity. In this paper we describe the isolation and structural elucidation of 1–5.

The sponge *Luffariella* sp.[7] was collected off Okinawa Island and kept frozen until used. The methanolic extract of the sponge was partitioned between toluene and water. The toluene solubles were subjected to repeated silica gel column chromatographies followed by reversed-phase or normal-phase HPLC to yield luffariolides A (1, 0.00075%, wet weight), B (2, 0.001%), C (3, 0.0015%), D (4, 0.0002%), and E (5, 0.0004%) as colorless oils, together with known compounds (6*E*)- and (6*Z*)-neomanoalides[2b] (6 and 7, 0.007% and 0.01%, respectively).

The molecular formulas of luffariolides A–E (1–5) were determined by HREIMS, respectively, and each of compounds 1–5 showed a characteristic EIMS fragment ion at $m/z$ 137, which was attributed to a $C_{10}H_{17}$ alkylated cyclohexenyl end group as observed in the EIMS of manoalide[2a] and neomanoalides.[2b] Luffariolide A (1) was




optically inactive, the IR and $^{13}C$ NMR data of 1 were indicative of the presence of an $\alpha,\beta$-unsaturated ester group [$\nu_{max}$ 1740 cm$^{-1}$; $\delta_C$ 174.0 (s)] and the UV absorption maximum at 325 nm ($\epsilon$ 25 000) suggested the presence of a trienone system. The $^1H$ NMR spectrum of 1 in $CDCl_3$ reveals signals due to five olefinic protons, two oxymethylenes, seven other methylenes, and four methyl groups. The $^{13}C$ NMR chemical shifts of the C-6–C-24 moiety of 1 (Table I) are nearly identical to those of (6*E*)-neomanoalide (6),[2b] indicating that 1 possesses a structure related to 6. The structural differences are found in the C-1–C-5 and C-25 segment. In the $^1H$–$^1H$ COSY spectrum of 1, H-2 and H-6 show allylic couplings to $H_2$-25 and $H_2$-24, respectively, and H-5 is coupled to H-4 and H-6. Since the $^1H$ signals due to the C-25 oxymethylene protons resonate at lower field ($\delta_H$ 5.00) than the C-24 oxymethylene protons ($\delta_H$ 4.22), the C-25 oxymethylene is assigned to the carbon bearing an ester oxygen. The remaining sp$^2$ carbon signals are ascribed to those of the trienone chromophore (C-2–C-6). NOE enhancements were observed for H-2(irradiated)/H-4(2%), $H_2$-24(irradiated)/H-6(4%), and $H_2$-25(irradiated)/H-5(5%), suggesting 2*Z*, 4*E*, and 6*E*-configurations. The $^{13}C$ chemical shift of C-23 ($\delta_C$ 16.2) implies the 10*E*-configuration. Thus, the structure of luffariolide A was concluded to be 1.

The UV [$\lambda_{max}$ 218 nm ($\epsilon$ 7400)] and IR ($\nu_{max}$ 1740 and 3380 cm$^{-1}$) data of luffariolide B (2) indicate the presence of butenolide and hydroxyl groups. The $^1H$ NMR spectrum of 2 is similar to that of (6*Z*)-neomanoalide (7) except for the signals for H-4 ($\delta_H$ 4.64) and $H_2$-25 ($\delta_H$ 4.87). The chemical shift differences of these $^1H$ signals from those of 7 (H-4 $\delta_H$ 5.09; $H_2$-25 $\delta_H$ 4.56 and 4.46) suggest that luffariolide B (2) possesses an ester linkage between C-1 and C-25. The $^{13}C$ NMR chemical shifts (Table I) of C-8 ($\delta_C$ 35.2) and C-24 ($\delta_C$ 60.2) of 2 indicate the 6*Z*-configuration. Thus, luffariolide B (2) was deduced to have a 4-hydroxy-1,25-olide group, i.e., a regioisomer of (6*Z*)-neomanoalide (7). The compounds corresponding to 2 with 4*S*- and 4*R*-configurations have been already reported as synthetic[8a] and degradation products of natural manoalide,[8b] respectively. The CD spectrum of 2 [232 nm ([$\theta$] 1800) and 218 ([$\theta$] 2700)] is the same as that of the 4*R*-isomer. The absolute configuration of the C-4 position of luffariolide B (2) is, therefore, assigned as *R*.

Luffariolide C (3) shows IR bands at 3300 and 1760 cm$^{-1}$, which are assignable to hydroxyl and $\gamma$-lactone groups, respectively. The $^{13}C$ signals due to C-4–C-25 of 3 (Table I) resemble those of (6*Z*)-neomanolide (7), while two sp$^3$ carbon signals are observed at $\delta_C$ 32.7 (t) and 41.5 (d) in place of two of the sp$^2$ carbon signals [$\delta_C$ 115.7 (C-2) and $\delta_C$ 173.1 (C-3)] observed for 7. The $^1H$ NMR spectrum also shows signals due to sp$^3$ methylene ($\delta_H$ 2.62 and 2.38) and sp$^3$ methine ($\delta_H$ 2.49) protons for the C-2–C-3 part of 3, and the $^1H$–$^1H$ COSY and HOHAHA spectra reveal the proton connectivities from $H_2$-2 to H-6. Thus, luffariolide C (3) was deduced to be 2,3-dihydro-(6*Z*)-neomanoalide (7). A homonuclear 2*D* *J*-resolution spectrum shows the coupling constant between H-3 and H-4 to be nearly 0 Hz, suggesting that the dihedral angle between H-3 and H-4 is almost 90°. NOESY correlations are observed for H-3/$H_2$-5 and H-4/H-25b. These observations indicate a 3,4-trans configuration for 3. Treatment of luffariolide B (2) with $NaBH_4$ afforded 2,3-dihydroluffariolide B (8) and luffariolide C (3) through a stereospecific 1,4-reduction (Scheme I). Spectral data including the optical rotation of 3 derived from luffariolide B (2) are consistent with those of natural luffariolide C (3). Since luffariolide B (2) was determined to possess the 4*R*-configuration, the absolute configuration of luffariolide C (3) was elucidated to be 3*R*,4*R*. Treatment of (6*Z*)-neomanoalide (7) with $NaBH_4$ also yielded luffariolide C (3) and compound 8, indicating that the neomanoalide also has the 4*R*-configuration.

Luffariolide D (4) shows IR bands at 3300 and 1770 cm$^{-1}$, suggesting the presence of hydroxyl and $\gamma$-lactone groups, respectively. The $^1H$ and $^{13}C$ NMR signals at $\delta_H$ 5.24 (br

(7) A voucher specimen of this sponge *Luffariella* sp. (SS-15) was deposited at Faculty of Pharmaceutical Sciences, Hokkaido University.

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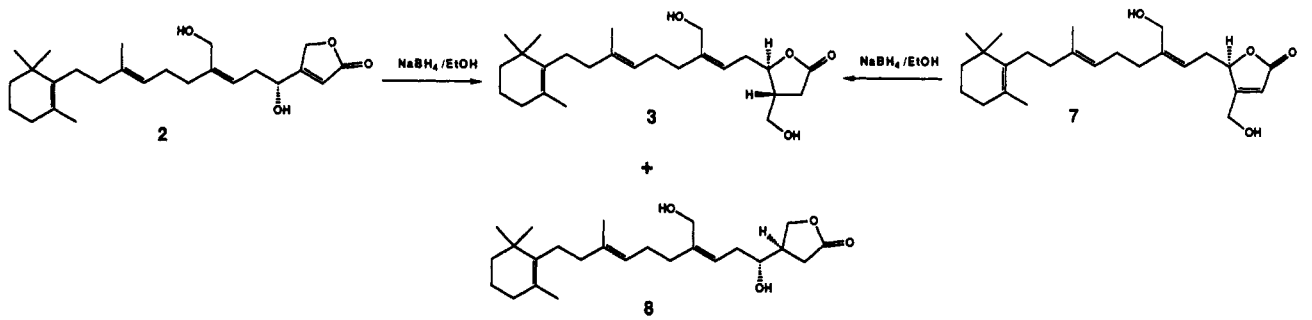
Table I. $^{13}C$ NMR Data of Luffariolides A–E (1–5) and (6*E*)- and (6*Z*)-Neomanoalides (6 and 7) in $CDCl_3$

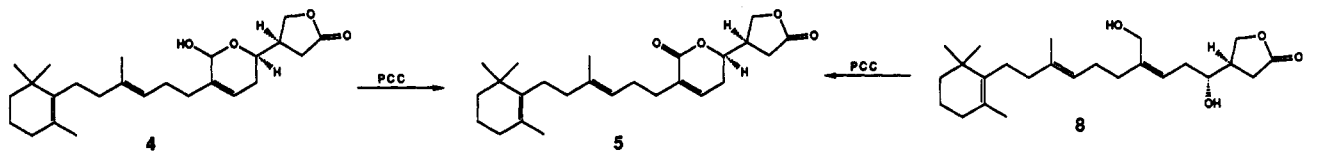
| | 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| position | δ[a] | m[b] | δ | m | δ | m | δ | m | δ | m | δ | m | δ | m |
| 1 | 174.0 | s | 173.6 | s[f] | 176.1 | s | 176.5 | s | 176.1 | s | 173.0 | s[j] | 172.3 | s[l] |
| 2 | 114.7 | d | 114.9 | d | 32.7 | t[g] | 30.9 | t | 30.2 | t | 115.9 | d[k] | 115.7 | d |
| 3 | 162.2 | s | 172.3 | s[f] | 41.5 | d | 39.7 | d | 39.7 | d | 172.2 | s[j] | 173.1 | s[l] |
| 4 | 132.8 | d | 67.4 | d | 82.8 | d | 67.6 | d | 77.4 | d | 81.8 | d | 82.0 | d |
| 5 | 123.3 | d[e] | 36.8 | t | 30.9 | t | 32.8 | t | 30.9 | t | 30.2 | t | 30.3 | t |
| 6 | 121.7 | d[e] | 120.0 | d | 121.7 | d | 121.4 | d | 137.4 | d | 117.1 | d[k] | 119.7 | d |
| 7 | 149.6 | s | 144.2 | s | 142.8 | s | 137.3 | s | 128.8 | s | 143.4 | s | 143.3 | s |
| 8 | 29.2 | t | 35.2 | t | 35.8 | t | 39.9 | t[h] | 39.8 | t[i] | 28.5 | t | 35.4 | t |
| 9 | 27.8 | t | 27.9 | t | 27.8 | t | 28.0 | t | 28.0 | t | 27.8 | t | 27.9 | t |
| 10 | 122.1 | d[e] | 122.8 | d | 123.0 | d | 122.9 | d | 122.7 | d | 122.7 | d | 122.9 | d |
| 11[c] | 136.9 | s | 137.0 | s | 137.1 | s | 137.1 | s | 137.4 | s | 137.1 | s | 136.9 | s |
| 12[d] | 39.8 | t | 39.8 | t | 39.8 | t | 39.9 | t[h] | 39.8 | t[i] | 39.8 | t | 39.8 | t |
| 13 | 27.4 | t | 26.8 | t | 26.7 | t | 26.1 | t | 26.5 | t | 26.7 | t | 26.7 | 1 |
| 14[c] | 137.8 | s | 137.2 | s | 136.9 | s | 136.9 | s | 137.9 | s | 137.0 | s | 137.1 | s |
| 15 | 127.2 | s | 127.1 | s | 127.0 | s | 127.0 | s | 127.1 | s | 127.0 | s | 126.9 | s |
| 16 | 32.8 | t | 32.7 | t | 32.6 | t[g] | 32.7 | t | 32.8 | t | 32.7 | t | 32.7 | t |
| 17 | 19.8 | t | 19.5 | t | 19.5 | t | 19.6 | t | 19.6 | t | 19.5 | t | 19.5 | t |
| 18[d] | 40.2 | t | 40.2 | t | 40.3 | t | 40.3 | t[h] | 40.3 | t[i] | 40.2 | t | 40.2 | t |
| 19 | 35.0 | s | 35.0 | s | 35.0 | s | 35.0 | s | 35.0 | s | 34.9 | s | 34.9 | s |
| 20, 21 | 28.6 | q | 28.6 | q | 28.6 | q | 28.7 | q | 28.7 | q | 28.6 | q | 28.6 | q |
| 22 | 19.5 | q | 19.8 | q | 19.8 | q | 19.8 | q | 19.8 | q | 19.8 | q | 19.8 | q |
| 23 | 16.2 | q | 16.2 | q | 16.1 | q | 16.1 | q | 16.2 | q | 16.1 | q | 16.0 | q |
| 24 | 66.1 | t | 60.2 | t | 63.2 | t | 91.4 | d | 163.1 | s | 66.1 | t | 60.0 | t |
| 25 | 70.5 | t | 71.3 | t | 60.2 | t | 70.3 | t | 69.7 | t | 58.5 | t | 58.4 | t |

[a] δ in ppm. [b] Multiplicity in DEPT. [c–l] Signals may be interchangeable.

**Scheme I**




**Scheme II**




s) and $\delta_C$ 91.4 (d) of **4** are reminiscent of the hemiacetal functionality found in manoalide.[2] The $^{13}C$ chemical shifts of the δ-lactol ring [$\delta_C$ 63.3 (d, C-4), 33.1 (t, C-5), 121.1 (d, C-6), 137.7 (s, C-7), and 91.7 (d, C-24)] of manoalide[2] are consistent with those of **4** [$\delta_C$ 67.6 (d, C-4), 32.8 (t, C-5), 121.4 (d, C-6), 137.3 (s, C-7), and 91.4 (d, C-24)]. The presence of a γ-lactone ring attached to C-4 was deduced from the $^1H$–$^1H$ COSY spectrum. H-4 shows a correlation to H-3, and H-3 showed cross peaks to $H_2$-25 and $H_2$-2. The $^1H$ chemical shifts implied that $H_2$-25 is attached to a methylene carbon bearing an ester oxygen and $H_2$-2 is adjacent to a carbonyl group. The 2D *J*-resolution spectrum reveals that H-4 is coupled to $H_2$-5 by $J$ = 14.8 and 6.8 Hz, indicating that H-4 is axially oriented. In addition, this spectrum shows the coupling constant between H-3 and H-4 is nearly 0 Hz, suggesting that the dihedral angle between H-3 and H-4 is almost 90°. Irradiation of H-4 yielded NOE (7.5%) for H-2b. The two possible Newman projections that could account for these observations are shown in Figure 1. Model a would be unlikely because of steric hindrance between $H_2$-5 and $H_2$-25. The relative

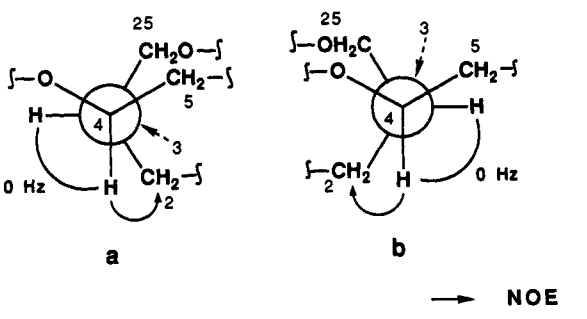



Figure 1. Newman projections of compound **4**.

configurations of H-3 and H-4 of luffariolide D (**4**) are, therefore, assigned as depicted in the projection b. This assignment as well as the absolute configuration was clarified by chemical correlation with 2,3-dihydroluffariolide B (**8**) and luffariolide E (**5**) (vide infra, Scheme II).

The UV [230 nm (ε 9600)] and IR [1715 and 1775 $cm^{-1}$] data of luffariolide E (**5**) suggest the presence of α,β-un-

saturated δ-lactone and γ-lactone groups. The $^1H$ and $^{13}C$ NMR spectra of **5** are similar to those of luffariolide D (**4**), and the prominent difference is the presence of another lactone carbonyl [$\delta_C$ 163.1, (s)] for compound **5** in place of the hemiacetal group [$\delta_C$ 91.4 (d); $\delta_H$ 5.24 (br s)] for compound **4**. The signal due to H-6 for **5** resonates at a low field ($\delta_H$ 6.58), which is assigned to the β-proton on a conjugated δ-lactone ring. Thus, luffariolide E (**5**) was suggested to be the 24-dehydro analog of luffariolide D (**4**). This was confirmed by conversion of luffariolide D (**4**) into luffariolide E (**5**) through PCC oxidation (Scheme II). Treatment of (3*R*,4*R*)-2,3-dihydroluffariolide B (**8**), which was obtained by $NaBH_4$ reduction of luffariolide B (**2**), with PCC also afforded luffariolide E (**5**), whose optical rotation and other spectral data are completely consistent with those of natural luffariolide E (**5**). Thus, the absolute configurations of **4** and **5** were determined to be 3*R* and 4*R*.

Luffariolides (**1**–**5**) and neomanoalides (**6** and **7**) exhibited cytotoxic activities against murine lymphoma L1210 cells in vitro with $IC_{50}$ values of 1.1, 1.3, 7.8, 4.2, 1.2, 9.8, and 5.6 μg/mL, respectively.[9]

## Experimental Section

**General Methods.** $^1H$ and $^{13}C$ NMR spectra were recorded on JEOL JMN GX-270 and EX-400 spectrometers. EI mass spectra were obtained operating at 70 eV.

**Collection, Extraction, and Isolation.** The yellowish brown sponge of the genus *Luffariella* was collected off Okinawa Island and kept frozen until used. The sponge (1.0 kg, wet weight) was extracted twice with MeOH (1.2 and 1 L) and then evaporated to give a residue (33.2 g). The residue was dissolved with toluene/MeOH (1:3) and partitioned between toluene (500 mL × 2) and 1 M NaCl (500 mL). The toluene-soluble portion was evaporated under reduced pressure to give a crude residue (2.99 g), which was subjected to silica gel column chromatography (Wako gel C-300, Wako Pure Chemical 4.6 × 43 cm) with $CHCl_3$/MeOH (100:0–85:15). The fraction eluting with 95% $CHCl_3$/MeOH was rechromatographed on a silica gel column (2.3 × 43 cm) with hexane/EtOAc (1:1) and by reversed-phase HPLC (Develosil ODS-5, Nomura Chemical, 10 × 250 mm; flow rate 2.5 mL/min; RI and UV (230 nm) detection; eluent MeOH/$H_2O$ (90:10)) to afford luffariolides A (**1**, 7 mg, 0.0007%, wet weight, $t_R$ 13.8 min), D (**4**, 1.9 mg, 0.000 19%, $t_R$ 20.0), and crude luffariolide E ($t_R$ 16.0), which was finally separated by a silica gel column (0.5 × 12 cm) with benzene/acetone (95:5) to give luffariolide E (**5**, 3 mg, 0.0003%). The 85% $CHCl_3$/MeOH eluate was rechromatographed on a silica gel column (2.3 × 40 cm) with hexane/EtOAc (3:7–1:9) to give (*Z*)- and (*E*)-neomanoalides (**6** and **7**, 70 and 130 mg, 0.007% and 0.013%, respectively) as major components. The hexane/EtOAc (3:7) eluate was further purified by a silica gel column (1.0 × 10 cm) with hexane/acetone (4:1) to give luffariolide B (**2**, 10 mg, 0.001%). The hexane/EtOAc (1:9) eluate was separated by a silica gel column [1.0 × 18 cm; hexane/acetone (3:1)] followed by HPLC on silica gel (Senshu Pak Silica-4251-S, Senshu Scientific, 10 × 250 mm, flow rate 4 mL/min; RI detection; eluent, EtOAc) to afford luffariolide C (**3**, 16 mg, 0.0016%).

**Luffariolide A (1)**: colorless oil; IR (neat) $\nu_{max}$ 3400, 2930, 2880, 1740, and 1610 $cm^{-1}$; UV (EtOH) $\lambda_{max}$ 325 nm (ε 25 000); $^1H$ NMR ($CDCl_3$) $\delta_H$ 0.97 (6 H, s, $H_3$-20 and $H_3$-21), 1.39 (2 H, m, $H_2$-18), 1.56 (2 H, m, $H_2$-17), 1.57 (3 H, s, $H_3$-22), 1.64 (3 H, s, $H_3$-23), 1.89 (2 H, t, *J* = 6.0 Hz, $H_2$-16), 1.94 (4 H, m, $H_2$-12 and $H_2$-13), 2.17 (2 H, br t, *J* = 6.9 Hz, $H_2$-9), 2.31 (2 H, br t, *J* = 6.9 Hz, $H_2$-8), 4.22 (2 H, br s, $H_2$-24), 5.00 (2 H, br s, $H_2$-25), 5.13 (1 H, br t, *J* = 6.9 Hz, H-10), 5.91 (1 H, br s, H-2), 6.28 (1 H, d, *J* = 11.0 Hz, H-6), 6.48 (1 H, d, *J* = 15.9 Hz, H-4), and 6.80 (1 H, dd, *J* = 11.0 and 15.9 Hz, H-5); EIMS *m/z* 384 ($M^+$, 5), 368 ($M - H_2O$, 1)$^+$, 180 (42), and 137 (100); HREIMS *m/z* 384.2679 ($M^+$, calcd for $C_{25}H_{36}O_3$, 384.2665).

**Luffariolide B (2)**: colorless oil; $[\alpha]_D^{25}$ +20° (*c* 1.0, $CHCl_3$); IR (neat) $\nu_{max}$ 3380, 2930, 2880, 1740, 1440, and 1020 $cm^{-1}$; UV (EtOH) $\lambda_{max}$ 218 nm (ε 7400); $^1H$ NMR ($CDCl_3$) $\delta_H$ 0.98 (6 H, s, $H_3$-20 and $H_3$-21), 1.41 (2 H, m, $H_2$-18), 1.56 (2 H, m, $H_2$-17), 1.59 (3 H, s, $H_3$-23), 1.64 (3 H, br s, $H_3$-23), 1.90 (2 H, t, *J* = 6.2 Hz, $H_2$-16), 2.02 (4 H, br s, $H_2$-12 and $H_2$-13), 2.17 (4 H, br s, $H_2$-8 and $H_2$-9), 2.53 (2 H, tt, *J* = 6.2 and 8.4 Hz, $H_2$-5), 4.08 (1 H, d, *J* = 10.2 Hz, H-24a), 4.17 (1 H, d, *J* = 10.2 Hz, H-24b), 4.64 (1 H, t, *J* = 6.2 Hz, H-4), 4.87 (2 H, br s, $H_2$-25), 5.12 (1 H, t, *J* = 6.2 Hz, H-10), 5.38 (1 H, t, *J* = 8.4 Hz, H-6), and 5.97 (1 H, d, *J* = 1.5 Hz, H-2); EIMS *m/z* 402 ($M^+$, 1), 278 (1.5), and 137 (100); HREIMS *m/z* 402.2753 ($M^+$, calcd for $C_{25}H_{38}O_4$, 402.2770).

**Luffariolide C (3)**: colorless oil; $[\alpha]_D^{25}$ +4.4° (*c* 1.6, $CHCl_3$); IR (neat) 3300, 2930, 2880, 1760, 1440, 1375, 1355, 1195, and 1005 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta_H$ 0.99 (6 H, s, $H_3$-20 and $H_3$-21), 1.40 (2 H, m, $H_2$-18), 1.56 (2 H, m, $H_2$-17), 1.59 (3 H, s, $H_3$-22), 1.64 (3 H, br s, $H_3$-23), 1.90 (2 H, t, *J* = 6.2 Hz, $H_2$-16), 2.02 (4 H, m, $H_2$-12 and $H_2$-13), 2.18 (4 H, m, $H_2$-8 and $H_2$-9), 2.38 (1 H, dd, *J* = 7.3 and 15.7 Hz, H-2b), 2.49 (1 H, dddd, *J* = 4.8, 6.2, 7.3, and 7.7 Hz, H-3), 2.57 (2 H, m, $H_2$-5), 2.62 (1 H, dd, *J* = 7.7 and 15.7 Hz, H-2a), 3.64 (1 H, dd, *J* = 4.8 and 10.7 Hz, H-25b, 3.74 (1 H, dd, *J* = 6.2 and 10.7 Hz, H-25a), 4.08 (1 H, d, *J* = 12.9 Hz, H-24b) 4.17 (1 H, d, *J* = 12.9 Hz, H-24a), 4.44 (1 H, dd, *J* = 6.1 and 11.1 Hz, H-4), 5.13 (1 H, br t, *J* = 6.2 Hz, H-10), and 5.40 (1 H, t, *J* = 7.7 Hz, H-6); EIMS *m/z* 404 ($M^+$, 1), 386 (0.3), and 137 (100); HREIMS *m/z* 404.2938 ($M^+$, $C_{25}H_{40}O_4$, 404.2927).

**Luffariolide D (4)**: colorless oil; $[\alpha]_D^{20}$ +9.0° (*c* 0.15, $CHCl_3$); IR (neat) $\nu_{max}$ 3300, 2860, 1770, and 1010 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta_H$ 0.99 (6 H, s, $H_3$-20 and $H_3$-21), 1.41 (2 H, m, $H_2$-18), 1.56 (2 H, m, $H_2$-17), 1.60 (3 H, s, $H_3$-22), 1.64 (3 H, br s, $H_3$-23), 1.90 (2 H, t, *J* = 6.3 Hz, $H_2$-16), 1.98 (2 H, m, $H_2$-5), 2.03 (4 H, m, $H_2$-12 and $H_2$-13), 2.13 (4 H, m, $H_2$-8 and $H_2$-9), 2.40 (1 H, dd, *J* = 7.3 and 10.5 Hz, H-2b), 2.59 (1 H, dd, *J* = 8.3 and 10.5 Hz, H-2a), 2.64 (1 H, br s, 24-OH), 2.68 (1 H, dddd, *J* = 6.4, 7.3, 7.8, and 8.3 Hz, H-3), 3.95 (1 H, dd, *J* = 6.8 and 14.4 Hz, H-4), 4.30 (1 H, dd, *J* = 6.4 and 9.3 Hz, H-25b), 4.44 (1 H, dd, *J* = 7.8 and 9.3 Hz, H-25a), 5.13 (1 H, br t, *J* = 6.3 Hz, H-6), 5.24 (1 H, br s, H-24), and 5.66 (1 H, br t, *J* = 6.1 Hz, H-6); EIMS *m/z* 402 ($M^+$, 0.5), 384 (1), 369 (0.7), and 137 (100); HREIMS *m/z* 402.2777 ($M^+$, calcd for $C_{25}H_{48}O_4$, 402.2770).

**Luffariolide E (5)**: colorless oil; $[\alpha]_D^{17}$ +7.1° (*c* 0.42, $CHCl_3$); IR (neat) $\nu_{max}$ 2920, 2850, 1775, 1710, 1430, 1375, 1210, 1170, 1095, and 1015 $cm^{-1}$; UV (EtOH) $\lambda_{max}$ 230 (ε 7500); $^1H$ NMR ($CDCl_3$) $\delta_H$ 0.99 (6 H, s, $H_3$-20 and $H_3$-21), 1.41 (2 H, dd, *J* = 3.5 and 6.1 Hz, $H_2$-18), 1.56 (2 H, m, $H_2$-17), 1.60 (3 H, s, $H_3$-22), 1.63 (3 H, s, $H_3$-23), 1.90 (2 H, t, *J* = 6.1 Hz, $H_2$-16), 2.02 (4 H, m, $H_2$-12 and $H_2$-13), 2.18 (2 H, m, $H_2$-9), 2.31 (1 H, m, H-8b), 2.34 (2 H, m, $H_2$-5), 2.38 (1 H, m, H-8a), 2.41 (1 H, dd, *J* = 17.6 and 8.6 Hz, H-2b), 2.63 (1 H, dd, *J* = 17.6 and 9.0 Hz, H-2a), 2.92 (1 H, br dt, *J* = 16.1 and 7.6 Hz, H-3), 4.33 (1 H, dd, *J* = 6.2 and 11.2 Hz, H-4), 4.34 (1 H, dd, *J* = 9.5 and 7.1 Hz, H-25b), 4.54 (1 H, dd, *J* = 9.5 and 7.8 Hz, H-25a), 5.11 (1 H, br t, *J* = 7.3 Hz, H-10), and 6.58 (1 H, m, H-6); EIMS *m/z* (%) 400 ($M^+$, 1.5), 386 (1), 276 (3), 264 (2), 196 (10), and 137 (100); HREIMS *m/z* 400.2655 ($M^+$, calcd for $C_{25}H_{36}O_4$, 400.2614).

**Reduction of Luffariolide B (2) with $NaBH_4$.** $NaBH_4$ (4.1 mg) in EtOH was cooled at 0 °C, and to this solution was added an EtOH solution (0.2 mL) of luffariolide B (**2**, 4.8 mg). The reaction mixture was stirred at room temperature for 2 h. After addition of water (2 mL) the aqueous layer was extracted with EtOAc (4 mL × 3). The organic layer was dried over anhydrous $MgSO_4$ and subjected to a silica gel HPLC (Senshu Pak Silica-1251-S, 4.6 × 250 mm, flow rate 2 mL/min; RI detection; eluent, EtOAc) to give luffariolide C [**3**, 1.8 mg, $[\alpha]_D^{17}$ +4.8° (*c* 0.1, $CHCl_3$)] and 2,3-dihydroluffariolide B (**8**, 1.0 mg): colorless oil; $[\alpha]_D^{17}$ +4.4° (*c* 0.1, $CHCl_3$); IR (neat) $\nu_{max}$ 3400, 2880, 1765, 1435, 1380, 1355, 1180, and 1010 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta_H$ 0.99 (6 H, s, $H_3$-20 and $H_3$-21), 1.41 (2 H, m, $H_2$-18), 1.56 (2 H, m, $H_2$-17), 1.59 (3 H, s, $H_3$-22), 1.64 (3 H, br s, $H_3$-23), 1.90 (2 H, t, *J* = 6.2 Hz, $H_2$-16), 2.02 (4 H, m, $H_2$-12 and $H_2$-13), 2.18 (4 H, m, $H_2$-8 and $H_2$-9), 2.28 (2 H, m, $H_2$-5), 2.38 (1 H, dd, *J* = 8.1 and 16.9 Hz, H-2b), 2.56 (1 H, dd, *J* = 8.8 and 16.9 Hz, H-2a), 2.64 (1 H, m, H-3), 3.60 (1 H, dd, *J* = 7.0 and 11.7 Hz, H-4), 4.05 (1 H, d, *J* = 11.4 Hz, H-24b),

(9) Luffariolides A (**1**), B (**2**), and E (**5**) were also cytotoxic against human carcinoma KB cells with $IC_{50}$ values of 4.7, 4.7, and 5.3 μg/mL, respectively, while luffariolides C (**3**) and D (**4**) and neomanoalides **6** and **7** showed weak cytotoxicities against KB cells, the inhibition at 10 μg/mL being 10.5%, 0.4%, 12.4%, and 18.9%, respectively.

4.20 (1 H, d, $J$ = 11.4 Hz, H-24a), 4.30 (1 H, dd, $J$ = 7.0 and 9.2 Hz, H-25b), 4.44 (1 H, dd, $J$ = 7.7 and 9.2 Hz, H-25a), 5.11 (br t, $J$ = 6.2 Hz, H-10), and 5.37 (1 H, t, $J$ = 8.4 Hz, H-6); EIMS $m/z$ 404 ($M^+$), 387, 371, and 137; HREIMS $m/z$ 404.2924 ($M^+$, $C_{25}H_{40}O_4$, 404.2926).

**Reduction of (6Z)-Neomanoalide (7) with $NaBH_4$.** To the cooled EtOH solution (1.5 mL) of $NaBH_4$ (10.2 mg) was added (Z)-neomanoalide (7, 18.9 mg) in 0.5 mL of EtOH and stirred at room temperature for 90 min. The same workup and purification as described above gave 3 [8.3 mg, $[\alpha]_D^{17}$ +3.9° (c 0.8, $CHCl_3$)] and 8 [3.4 mg, $[\alpha]_D^{17}$ +3.8° (c 0.3, $CHCl_3$)].

**Oxidation of 2,3-Dihydroluffariolide B (8) with PCC.** To a solution of 8 (2.0 mg) in $CH_2Cl_2$ (0.5 mL) was added PCC (4.5 mg) and powdered molecular sieves (4A, 8.9 mg), and the mixture was stirred at room temperature for 2 h. $Et_2O$ (5 mL) was added, and the reaction mixture was filtered with a membrane filter and washed with $Et_2O$ (5 mL × 2). The residue was subjected to a silica gel column (Wako gel C-300, 0.5 × 10 cm) with hexane/EtOAc (2:1) to give luffariolide E [5, 1.2 mg, $[\alpha]_D^{17}$ +9.8° (c 0.17, $CHCl_3$)].

**Oxidation of Luffariolide D (4) with PCC.** To the $CH_2Cl_2$ solution (0.5 mL) of luffariolide D (4, 1.0 mg) was added molecular sieves (4A, 9.3 mg) and PCC (3.5 mg, and the mixture was stirred at room temperature for 1 h. $Et_2O$ (5 mL) was added to the reaction mixture, filtered with a membrane filter, and washed with $Et_2O$ (5 mL × 2). The filtrate was evaporated under reduced pressure, and the residue was chromatographed on a silica gel column (0.5 × 8 cm) with hexane/EtOAc (6:4) to afford luffariolide E [5, 0.7 mg, $[\alpha]_D^{17}$ +9° (c 0.07, $CHCl_3$)].

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**Supplementary Material Available:** EIMS, HREIMS, IR, UV, 2D J-resolution, and NOESY spectra of 1–5 and 2D NMR correlation data of 1–5 (24 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

## An Efficient, Highly Stereoselective Synthesis of (Z)-16α-Hydroxy-17-ethylidene Steroids

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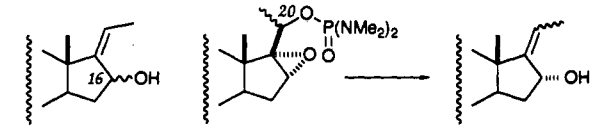
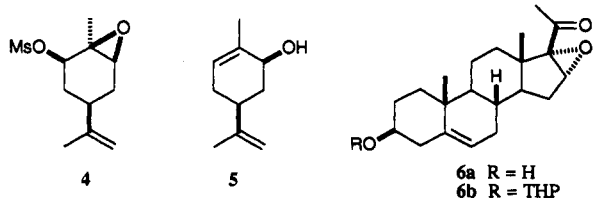
Both Z and E isomers of the 17-ethylidene derivatives of steroids and their 16-hydroxylated analogues serve as pivotal intermediates for the stereoselective introduction of steroid side chains.[1] While there are a number of multistep syntheses of these ethylidene compounds described in the literature,[2] the stereoselective synthesis of (Z)-16-hydroxy-17-ethylidene steroids 1 has remained problematic.[2,3] In the following paper, we show that such an ethylidene derivative can be synthesized efficiently, with complete stereoselectivity, by the use of a four-step sequence from the readily available 16α,17α-epoxypregnenolone (6a).

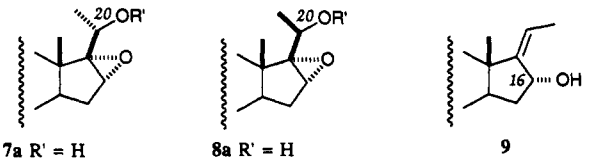
The present study was initiated with the aim of examining the effect of the stereochemistry at C-20 upon the stereochemical outcome of the reductive epoxide ring opening of epoxy phosphorodiamidates (see 2 → 3). The extent of the stereochemical preservation, if any, during the carbanion formation by dissolving metal reduction of a phosphorodiamidate, together with the timing of the epoxide-ring opening, were of great interest to us. In this context, it should be noted that Yamamoto reported, as a means for stereoselective 1,3-transposition of allylic alcohols, that a similar reduction of epoxy alcohol methanesulfonate 4 with Na–$NH_3$ or Na-naphthalene produced allylic alcohol 5 in high yield.[4] This overall syn elimination of the epoxy–mesylate unit seems to provide considerable mechanistic insight into the reaction, since, unlike acyclic 2,3-epoxy-1-alkanol mesylates, the reaction must proceed either by epoxide-ring opening of the *cis*-epoxy carbanion or possibly by that of the *trans*-epoxy carbanion.



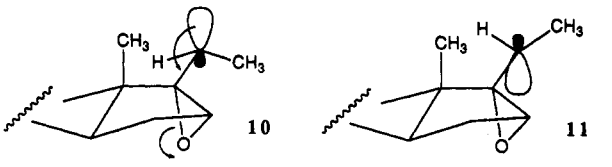

1 2 3




4 5 6a R = H 6b R = THP




7a R' = H 7b R' = P(=O)(NMe2)2 8a R' = H 8b R' = P(=O)(NMe2)2 9

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The requisite epoxy phosphorodiamidates 7b[5] and 8b were obtained in stereochemically pure form from the commercially available 16α,17α-epoxypregnenolone (6a). Thus, sodium borohydride reduction of epoxypregnenolone tetrahydropyranyl (THP) ether 6b,[3c,d,6] prepared from 6a with dihydropyran/p-TsOH/$CH_2Cl_2$ in 93% yield, provided quantitatively a 3:1 mixture of 20R- and 20S-epoxy alcohols, 7a and 8a, respectively. The stereochemical assignments of these alcohols were made by comparison of the proton NMR spectra of 7a and 8a with those re-

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(5) Structures 7–9 all share the same A–C ring structure as 6.

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