in DMSO and added dropwise to a solution of methylenetriphenylphosphine (made from dimsylsodium and methyltriphenylphosphonium bromide, 7.85 g, 0.022 mol, in DMSO[12]). The solution was stirred at room temperature for 2 h. The solution was diluted with an equal volume of water and extracted three times with pentane. The pentane solution was dried with $MgSO_4$ and the solvent removed under vacuum. After column chromatography and elution with petroleum ether, the product was obtained as an oil, yield 843 mg, 45%: ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.43 (2 H, s), 2.07 (4 H, s), 3.00 (4 H, d), 4.76 (4 H, s), 6.03 (2 H, s); MS 172.

3,6-Bis(methylene)cyclohexene (2). The product of the double Wittig reaction above (94 mg, 0.55 mmol) was added dropwise to a vertical pyrolysis tube packed with broken Pyrex tubing with a slow flow of nitrogen. The tube was held at 260 °C by a furnace. The lower end of the tube was connected to a trap containing pentane cooled by dry ice. After addition was complete and a volume of nitrogen several times the volume of the tube had passed, the trap was removed and maleic anhydride (54 mg, 0.55 mmol) dissolved in 1 mL acetone was added to remove the cyclopentadiene formed. The contents of the trap after warming to room temperature were then distilled to remove solvent and then subjected to preparative scale GC purification at about 75 °C. **2** was free from xylene and was isolated in 41% yield as found by analytical GC with an internal *p*-xylene standard: ^{1}H NMR (90 MHz, $CDCl_3$) δ 2.45 (4 H, s), 4.90 (4 H, s), 6.20 (2 H, s), the peak at 4.90 is just perceptibly split at 300 MHz; ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 30.30, 112.03, 130.49 (the quaternary carbons were not observed in this spectrum).

Isolation and Identification of Products from 1. Samples (about 500 mg) of the mixture of stereoisomers of **1** in mesitylene were heated in sealed evacuated tubes for typically about 1.5 h; very long heating times led to much reduced quantities of the isomers, short times gave isolable amounts of major products only (**2** was the major early product). The tubes were then opened and subjected to bulb-to bulb distillation, the first distillate contained the C_8H_{10} products with mesitylene, the later fraction contained mesitylene and the $C_{16}H_{20}$ materials. A preliminary preparative-scale GC on a short DEGS column gave a sample with very little mesitylene. On further GC **1**, **2**, **6**, and **4** were in that order eluted and trapped. The analytical "microbore" column also showed **5**, not isolated in these experiments. Compound **5** did not survive this chromatography, but it was later isolated in a mixture with **2** and the NMR spectrum was obtained by difference; it is given below.

An identical run using mesitylene-d_{12} as the solvent and analyzed on the GCMS showed products of parent mass 106 and 212 only, not distinguishable from products in ordinary mesitylene.

The samples were identified by NMR spectroscopy. The ^{1}H NMR spectrum of isolated **2** agrees with that for the synthetic sample above. The ^{1}H NMR spectral data of **6**, δ 2.30 (6 H, s), 7.04 (4 H, s) have been reported. Those of **4**, ^{1}H (300 MHz, $CDCl_3$) δ 1.55 (4 H, s), 2.00 (3 H, s), 5.70 (1 H, s), 6.00 and 6.40 (2 H, AB system), agree with the data reported;[13] ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 12.17, 15.55, 37.19, 132.63, 139.65, 140.08. A DEPT spectrum showed that only the 12.17 resonance was for carbon with two attached protons. The identifications of **2** and **6** were confirmed by comparison of GC retention with those of authentic samples. Diene **4** was further identified by a UV maximum at 261 nm compared to the reported[14] peak at 257 nm for the parent compound without the methyl. No structure was assigned to any dimer. Similar experiments in mesitylene but in far more dilute solution still showed substantial although somewhat less dimer. Experiments with added broken glass to the solution of **1** showed slightly larger relative amounts of **4**, suggesting a heterogeneous rearrangement of **5**.

The flash vacuum pyrolysis described below gave a sample of the retention time of **2** after preparative GC isolation; it gave two peaks on the analytical GC. The ^{1}H NMR (300 MHz, $CDCl_3$) of this mixture, after subtraction of the NMR peaks of **2**, gave a spectrum of the other smaller component, assigned to **5**: δ 0.80 (4 H, d), 2.65 (2 H, d), 4.85 (2 H, d), 5.70 and 6.10 (2 H, AB system). The quality of this difference spectrum did not allow evaluation of coupling constants. The characterization of **5** resulted from improved preparative GC technique which minimized rearrangement to **4**, not to the conditions of the experiment yielding it. **5** was present in the analytical GCs of all the reaction samples.

Flash vacuum pyrolysis allowed short exposure to much higher temperatures. A Vycor tube packed with broken glass tubing was heated by a horizontal tube furnace. At one end was a small flask containing **1** (ca. 20 mg) and an inlet for a slow flow of N_2. The other end led to a trap cooled in dry ice and then to a vacuum pump with the pressure controlled by a leak. The N_2 flow was started, the flask of **1** was immersed in liquid N_2, the pump was started, and when the pressure was stable the flask was allowed to warm, leading to quiet evaporation of **1**. The trap was warmed, and the contents were rinsed out with pentane for the analytical GC, which only showed peaks corresponding to **1**, **2**, **4**, **5**, and **6** at FVP temperatures up to 300 °C. At the higher temperatures three other minor products were found. One of these, an isomer of **1** by GCMS, appeared to come from **4** or **5**, because the amounts of these decreased as the new isomer increased. Amounts were too small for identification, although it was shown that the species was thermally stable in solution up to 162 °C for 20 h, by noting that the mixture isolated from FVP after most of the **1** had disappeared showed no decrease in the concentration of this new isomer compared to an internal standard. The other two were (by NMR) styrene and *p*-ethyltoluene, from more drastic reactions. No dimers were found, but a substantial amount of less volatile material was seen. Temperatures were determined by the furnace setting and checked before a run with a thermometer in the tube.

Kinetics of the Interconversion of the Stereoisomers of 1. Approximately 13-mg samples of the separated isomers (>98% stereochemically pure) were dissolved in 1 mL of mesitylene, with heptane as internal standard. Samples (20 μL) were sealed in small capillary tubes and immersed in a thermostated oil bath. Analytical GC of samples removed from time to time gave the extent of isomerization. The reactions were followed only to <5% completion, minimizing the further reactions and the reverse reaction. The resulting plots of extent of completion vs time gave the first-order rate constants summarized in Table I.

Acknowledgment. This work was supported by a grant from the Robert A. Welch Foundation. It is based on the PhD thesis of K.J.S., 1990. Thanks are due to Alan Kook for help with the NMR. The preparation of **1** is based on preliminary results from the group of Prof. W. E. Billups.

Supplementary Material Available: ^{1}H NMR spectra of 6-methylenespiro[2.4]hept-4-ene (**5**) and 3,6-bis(methylene)-*endo*-tricyclo[6.2.1.0]undec-9-ene (2 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.

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Alteramide A, a New Tetracyclic Alkaloid from a Bacterium *Alteromonas* sp. Associated with the Marine Sponge *Halichondria okadai*

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Marine microorganisms have proven to be a rich source of structurally novel and biologically active compounds

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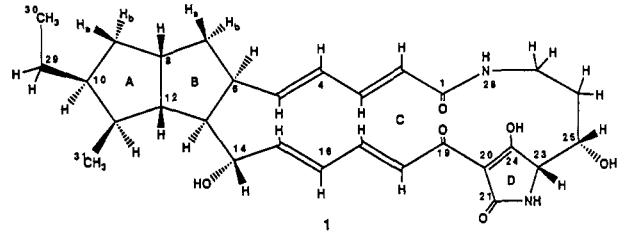
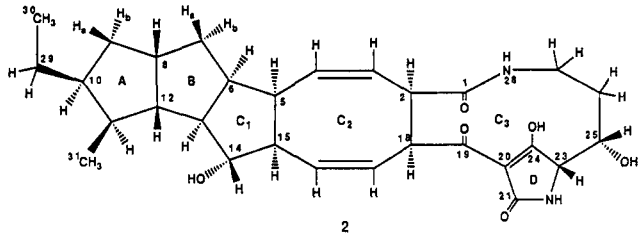
which might be useful for the development of new pharmaceutical agents.[2] In our search for bioactive compounds from marine microorganisms,[3] we have examined extracts of symbiotic bacteria associated with various marine invertebrates and isolated a new cytotoxic alkaloid, alteramide A (1), from the cultured mycelium of a bacterium *Alteromonas* sp. separated from the marine sponge *Halichondria okadai*. In this paper, we describe the isolation and structure elucidation of 1, which undergoes an intramolecular photochemical [4 + 4] cycloaddition to generate the hexacyclic derivative 2.



1

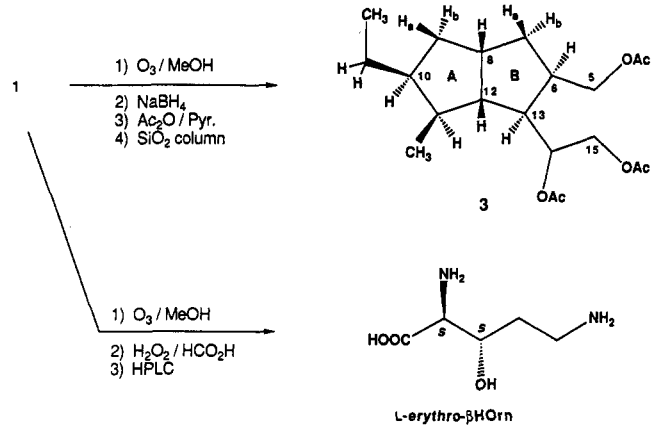


2

The bacterium *Alteromonas* sp. was isolated from the sponge *Halichondria okadai* collected off Nagai, Kanagawa, and grown statically in PYG broth [peptone 1%, yeast extract 0.5%, glucose 2.0%/50% ASW (artificial sea water)] at 25 °C for 7 days. The mycelium (52 g from 2 L of culture) was extracted with $CHCl_3$/MeOH (2:1), and the extract was purified repeatedly by a Sephadex LH-20 column (MeOH) followed by a Sep-Pak ODS cartridge (MeOH) to give alteramide A[4] (1, 0.1% wet weight) as a yellow powder.

The molecular formula of 1, $C_{29}H_{38}N_2O_6$, which requires 12 degrees of unsaturation, was established by the HRFABMS [m/z 533.2629 $(M + Na)^+$, Δ +0.1 mmu, $C_{29}H_{38}N_2O_6Na$]. The UV absorption at λ_{max} 347 nm (ϵ 11 000) indicated the presence of extended π conjugation. The IR spectrum was indicative of the presence of NH and/or OH (3400 cm^{-1}) and unsaturated carbonyls (1680 and 1610 cm^{-1}). The presence of a tetramic acid was suggested by ^{13}C NMR data (δ 194.9, 184.8, 180.0, and 103.1).[5] A conjugated amide carbon was observed at δ 168.9 and eight sp^2 methine carbons observed from δ 123.4 to 147.9 were assigned to four disubstituted double bonds. These assignments accounted for 9 of the 12 degrees of unsaturation, indicating that 1 contained a three-ring system.

The assignments of all of the protonated carbons were made by a HMQC spectrum.[6] Detailed analyses of the ^1H–^1H COSY spectrum of 1 revealed the presence of two segments, C-2–C-18 and N-22–N-28. Coupling constants (ca. 15 Hz each) between olefinic protons (H-2/H-3, H-4/H-5, H-15/H-16, and H-17/H-18) indicated all *E* geometries for the four double bonds. The connection between C-10 and C-11 was obscure in the ^1H–^1H COSY spectrum, but was clearly revealed by the HMBC spectrum[7] (cross peaks: H-11/C-10 and Me-31/C-10). The ^1H–^1H COSY spectrum of 1 showed a cross peak between H-8 and H-12, while the HMBC spectrum showed correlations of H-12/C-8, H-7a/C-12, and H-9a/C-12. These data suggested a linkage between C-8 and C-12 to form a five-membered ring system (ring A). The connection from C-6 to C-13 was revealed by the HMBC spectrum (cross peaks: H-5/C-13, H-6/C-13, H-7a/C-13, H-12/C-6, H-13/C-6, and H-14/C-6). From these results, it was established that a bicyclo[3.3.0]octane unit existed in 1. The connection from C-18 to C-25 through the tetramic acid was verified by the HMBC spectrum of 1 (cross peaks: H-18/C-19, H-23/C-21, H-23/C-24, H-23/C-25, and H-25/C-23). The HMBC spectrum showed three-bond couplings of H-3/C-1 and H-27b/C-1. These data established the connection from C-2 to C-27 through the amide bond (C-1–N-28). The relative stereochemistry of the bicyclo[3.3.0] unit was confirmed by NOE data of compound 3 derived from 1 through reductive ozonolysis and acetylation.[8] The correlations of H-8/H-12, H-8/H-9a, H-8/Me-31, Me-31/H-29a, and Me-31/H-29b observed in the NOESY spectrum of 3 established that those protons were located at the same side of the ring A and suggested that ethyl (C-29 and C-30) and methyl (C-31) groups were cis-oriented. Ring A and ring B were shown to be cis-fused from the NOESY correlations observed for H-8/H-12 and H-11/H-13. The NOESY correlation between H-6 and H-13 indicated that these protons were cis-oriented. The absolute stereochemistry at C-23 and C-25 was established as follows. Ozonolysis of 1 followed by oxidation with H_2O_2–HCO_2H gave β-hydroxyornithine (βHOrn). The chiral HPLC analysis clarified that the βHOrn had L-erythro configuration. Thus, the structure of alteramide A was concluded to be 1.



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(4) The sample used for NMR experiments contained ca. 20% of a minor component, alteramide B, which has the same ring system as that of alteramide A (1) without a hydroxyl group at C-25. However, it was difficult to separate alteramide A from alteramide B, since the sample gave a broad peak in HPLC (silica gel and ODS) under any solvent systems examined.

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Table I. ^1H and ^{13}C NMR Data of Alteramide A (1)[a]

position	H	J (Hz)	C	H coupled with C (HMBC correlations)
1			168.9, s	H-2, H-3, H-27b
2	5.78, d	15.1	123.4, d	H-4
3	6.85, dd	11.2, 15.1	141.9, d	H-4, H-5
4	6.04, dd	11.2, 14.9	129.9, d	H-2, H-3
5	5.60, dd	9.6, 14.9	147.9, d	H-3, H-7b, H-13
6	2.46, m		54.5, d	H-4, H-5, H-7a, H-7b, H-12, H-13, H-14
7a	1.95, m		44.2, t	H-6, H-8, H-12
7b	1.26, m			
8	2.42, m		42.8, d	H-7a, H-7b, H-9a, H-9b, H-12
9a	2.16, m		40.2, t	H-7b, H-10, H-29a, H-29b
9b	0.96, m			
10	1.50, m		54.5, d	H-9b, H-11, H-12, H-29b, H_3-30, H_3-31
11	1.36, m		48.5, d	H-9a, H-9b, H-12, H-13, H_3-31
12	2.14, m		58.8, d	H-7a, H-9a, H-11, H-13, H_3-31
13	1.62, m		58.0, d	H-5, H-6, H-7a, H-11, H-14
14	3.89, m		78.8, d	H-12, H-13, H-16
15	5.71, dd	8.3, 15.4	145.1, d	H-13, H-17
16	6.11, dd	10.7, 15.4	133.2, d	H-14, H-17, H-18
17	7.21, dd	10.7, 15.1	141.5, d	H-15, H-16, H-18
18	7.37, d	15.1	130.4, d	H-16, H-17
19			184.8, s	H-17, H-18
20			103.1, s	
21			180.1, s	H-23
22				
23	3.82, brs		68.7, d	H-25
24			194.9, s	H-23
25	3.98, m		72.6, d	H-23, H-27b
26a	1.40, m		32.7, t	H-23, H-25, H-27b
26b	1.30, m			
27a	3.70, m		38.1, t	H-25
27b	2.84, m			
28				
29a	1.68, m		27.9, t	H-11, H_3-30
29b	1.15, m			
30	0.95, t	7.0	12.8, q	H-29b
31	1.10, d	7.0	18.4, q	

[a] Recorded on a JEOL EX-400 spectrometer in CD_3OD.

Interestingly, alteramide A (1) was converted into the hexacyclic derivative 2 in MeOH solution under daylight at room temperature for 2 days. The molecular formula, $C_{29}H_{38}N_2O_6$, of 2 determined by the HRFABMS (m/z 533.2624, Δ −0.4 mmu, $C_{29}H_{38}N_2O_6Na$), was the same as that of 1. The NMR spectral differences between 1 and 2 were observed in the C-1–C-5 and C-15–C-19 segments. Resonances in the ^1H and ^{13}C NMR spectra of 2 indicated the presence of two double bonds (δ_H 6.16, 5.69, 5.57, and 5.41; δ_C 136.9, 136.9, 129.6, and 128.6). The coupling constants ($J_{3,4}$ = 8.8 Hz and $J_{16,17}$ = 9.3 Hz) indicated that the two double bounds were Z configurations. The ^1H–^1H COSY spectrum of 2 indicated the presence of carbon–carbon bonds from C-2 to C-18 and from C-5 to C-15 (cross peaks: H-2/H-18 and H-5/H-15). This suggested that a cyclooctadiene unit existed in 2. From these results 2 was concluded to be an intramolecular [4 + 4] cycloaddition product of 1. The relative stereochemistry of 2 was established by the ROESY spectrum.[9] The correlations of H-2/H-18, H-5/H-15, and H-5/H-6 indicated that the five-membered ring (ring C_1) and the eight-membered ring (ring C_2) were generated by cis-fusion, and the ring C_2 and the 11-membered ring (ring C_3) also by cis-fusion. It was suggested that the ring C_2 had a boat form since the correlations of H-2/H-5 and H-15/H-18 were observed in the ROESY spectrum. The configuration of the hydroxyl group at C-14 was elucidated to be α from the correlations of H-12/H-14 and H-14/H-16 in the ROESY spectrum. Compound 2 was also generated from 1 under irradiation of light at 0 °C for 30 min.

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Alteramide A (1) is a new macrocyclic lactam with a dienone and a dienoyltetramic acid which are susceptible to a unique intramolecular photochemical [4 + 4] cycloaddition to generate the hexacyclic product 2. It is noted that a marine bacterium *Alteromonas* sp. symbiotically associated with the sponge *Halichondria okadai* produces compound 1, since the related macrocyclic lactams were previously isolated from a terrestrial actinomycete[5a] and another marine sponge.[5b] Alteramide A (1) exhibited cytotoxicity against murine leukemia P388 cells, murine lymphoma L1210 cells, the human epidermoid carcinoma KB cells in vitro with the IC_{50} values of 0.1, 1.7, and 5.0 μg/mL, respectively, while compound 2 showed no cytotoxicity.

Experimental Section

General Procedure. ^1H and ^{13}C NMR spectra were recorded on a JEOL EX-400 spectrometer. The 3.35 ppm resonance of residual CHD_2OD and 49.0 ppm of CD_3OD were used as internal references, respectively. FABMS spectra were obtained on a JEOL HX-110 spectrometer by using glycerol as a matrix, and EIMS spectra were obtained on a JEOL DX-303 operating at 70 eV.

Collection and Cultivation. The bacterium *Alteromonas* sp. was isolated from the sponge *Halichondria okadai* which was collected at Nagai, Kanagawa, Japan. Cultures of *Alteromonas* sp. were grown in PYG broth [peptone (Diffco) 1%, yeast extract (Diffco) 0.5%, glucose 2% in 50% ASW (Jamarin S, Jamarin Laboratory), pH 7.0]. Cultures were incubated statically at 25 °C for 7 days. The cells were harvested by centrifugation (5000 rpm, 10 min.).

Isolation. The mycelium (52 g, wet weight) from 2 L of culture was extracted with $CHCl_3$/MeOH (2:1, 100 mL × 3) and evaporated under reduced pressure. The extract was subjected repeatedly to a Sephadex LH-20 column (3.5 × 28 cm) using MeOH.

The fraction eluting from 150 to 250 mL was subjected to chromatography on a Sep-Pak ODS cartridge (Waters) with MeOH/H_2O (3:7, 20 mL) and then MeOH (20 mL) to give alteramide (1, 50 mg, 0.1% wet weight) in the latter fraction.

Alteramide A (1): yellow powder; mp ~200 °C dec; $[\alpha]_D^{22}$ +36.2° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 268 (ϵ 30 300) and 347 nm (11 000); IR (KBr) ν_{max} 3400, 1660 (sh), 1610, and 1470 cm^{-1}; ^1H and ^{13}C NMR (Table I); FABMS (positive ion, glycerol matrix) *m/z* 533 (M^+ + Na); HRFABMS *m/z* 533.2629 (M^+ + Na; calcd for $C_{29}H_{38}N_2O_6Na$, 533.2628).

Photochemical Cycloaddition of 1. The irradiation was carried out using a mercury lamp housed in a water-cooled Pyrex jacket. A solution of 1 (3.0 mg) in 1.0 mL of degassed methanol was irradiated under argon atmosphere at 0 °C for 30 min. Removal of the methanol by evaporation under reduced pressure afforded 3.0 mg of **2**: white amorphous solid; mp ~160 °C dec; $[\alpha]^{22}_D$ + 16.7° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 245 (ϵ 15 000) and 286 nm (15 500); IR (KBr) ν_{max} 3330, 1640, 1520, and 1480 cm^{-1}; ^1H NMR (CD_3OD) δ 0.90 (3 H, t, *J* = 7.3 Hz, Me-30), 0.92 (1 H, m, H-7b), 0.94 (1 H, m, H-9b), 1.04 (3 H, d, *J* = 6.4 Hz, Me-31), 1.10 (1 H, m, H-29b), 1.26 (1 H, m, H-11), 1.28 (1 H, m, H-26b), 1.34 (m, H-26a), 1.37 (1 H, m, H-10), 1.64 (1 H, m, H-29a), 1.66 (1 H, m, H-12), 1.80 (2 H, m, H-6, H-13), 1.92 (1 H, m, H-7a), 2.08 (1 H, m, H-9a), 2.76 (1 H, m, H-8), 2.86 (1 H, m, H-5), 3.00 (1 H, m, H-27b), 3.20 (1 H, m, H-27a), 3.26 (1 H, m, H-15), 3.60 (1 H, m, H-14), 4.00 (1 H, m, H-2), 4.03 (1 H, m, br s, H-23), 4.10 (1 H, m, H-25), 5.12 (1 H, m, H-18), 5.41 (1 H, dd, *J* = 5.3, 8.8 Hz, H-4), 5.57 (1 H, dd, *J* = 8.1, 8.8 Hz, H-3), 5.69 (1 H, dd, *J* = 8.3, 9.3, H-16), and 6.16 (1 H, m, H-17); ^{13}C NMR (CD_3OD) δ 13.0 (q, C-30), 18.5 (q, C-31), 27.2 (t, C-29), 30.7, (t, C-26), 36.6 (t, C-27), 36.7 (t, C-7), 41.6 (t, C-9), 45.8 (d, C-5), 47.8 (d, C-11, C-18), 50.4 (d, C-8), 51.1 (d, C-2), 54.2 (d, C-12), 54.9 (d, C-10), 58.8 (d, C-6), 59.6 (d, C-15), 65.2 (d, C-13, C-23), 78.2 (d, C-25), 81.9 (d, C-14), 108.1 (s, C-20), 128.6 (d, C-17), 129.6 (d, C-3), 135.9 (d, C-16), 136.9 (d, C-4), 175.7 (s, C-1), 176.8 (s, C-21), and 191.1 (s, C-19, C-24). FABMS (positive ion, glycerol matrix) *m/z* 533 (M + Na)$^+$; HRFABMS *m/z* 533.2624 (M^+ + Na; calcd for $C_{29}H_{38}N_2O_6Na$, 533.2628).

Reductive Ozonolysis of 1. Through a solution of 1 (9.0 mg) in MeOH (1.0 mL) at −40 °C was passed ozonized oxygen for 5 min. After the excess of ozone was expelled by a nitrogen stream, the mixture was allowed to warm to 0 °C and $NaBH_4$ (9.0 mg) was added. The mixture was stirred for 45 min, and excess hydride was decomposed with 1 mL of 1 M potassium phosphate buffer (pH 7). The mixture was extracted with EtOAc (1 mL × 5), and the organic solvent was evaporated. The residue was treated with acetic anhydride/pyridine (1:1) overnight at room temperature. After evaporation under reduced pressure the residue was purified by chromatography on a silica gel column (0.7 × 7 cm) eluted with hexane/acetone (10:1) to yield compound **3** (3.0 mg) as a colorless oil: $[\alpha]_D^{22}$ −25.3° (*c* 0.6, $CHCl_3$); IR (neat) ν_{max} 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.86 (3 H, t, *J* = 7.6 Hz, Me-30), 0.96 (3 H, d, *J* = 6.8 Hz, Me-31), 0.98 (1 H, m, H-9b), 1.06 (1 H, m, H-29b), 1.10 (1 H, m, H-7b), 1.20 (1 H, m, H-11), 1.34 (1 H, m, H-10), 1.60 (1 H, m, H-29a), 1.79 (1 H, m, H-13), 1.86 (1 H, m, H-12), 2.01 (1 H, dt, *J* = 7.3, 11.7 Hz, H-7a), 2.04 (3 H, s, CH_3CO), 2.05 (3 H, S, CH_3CO), 2.06 (3 H, s, CH_3CO), 2.09 (1 H, m, H-9a), 2.11 (1 H, m, H-6), 2.34 (1 H, m, H-8), 4.03 (1 H, dd, *J* = 5.9, 10.7 Hz, H-5′), 4.05 (1 H, dd, *J* = 7.8, 11.7 Hz, H-15′), 4.09 (1 H, dd, *J* = 5.9, 10.7 Hz, H-5), 4.28 (1 H, dd, *J* = 3.9, 11.7 Hz, H-15), and 5.16 (1 H, dd, *J* = 3.9, 7.8 Hz, H-14); ^{13}C NMR ($CDCl_3$) δ 12.4 (q, C-30), 17.1 (q, C-31), 20.8 (q, CH_3CO), 20.9 (q, CH_3CO), 21.1 (q, CH_3CO), 26.8 (t, C-29), 37.5 (t, C-9), 38.9 (t, C-7), 41.9 (d, C-8), 43.7 (d, C-6), 46.2 (d, C-11), 48.2 (d, H-13), 51.5 (d, H-10), 55.5 (d, C-12), 64.4 (t, C-15), 67.4 (t, C-5), 73.2 (d, C-14), 170.6 (s, CH_3CO), 170.7 (s, CH_3CO), and 171.1 (s, CH_3CO); EIMS *m/z* 309 ($M^+ - CH_3COO$), and 248 ($M^+ - 2 \times CH_3COOH$); HREIMS *m/z* 309.2094 [($M^+ - CH_3COO$); calcd for $C_{18}H_{29}O_4$, 309.2065].

Determination of L-*erythro*-β-Hydroxyornithine. Through a solution of 1 (2.0 mg) in MeOH (0.7 mL) at −40 °C was passed ozonized oxygen for 2 min. After the excess of ozone was expelled by a nitrogen stream, the solution was evaporated under reduced pressure, and to the residue 98% formic acid (0.5 mL) and 35% hydrogen peroxide (50 μL) were added. The mixture was stirred for 1 h at 0 °C and then for 18 h at room temperature. The solvent was evaporated, and the residue was chromatographed on a Sep-Pak ODS cartridge with MeOH/H_2O (3:7) to give a mixture of amino acids (0.4 mg), which was analyzed by a Hitachi amino acid autoanalyzer (Model 835; No. 2617, 4.0 × 250 mm) at a flow rate of 0.275 mL/min with 0.2 N sodium citrate buffer and detected at 570 nm. The relative stereochemistry of βHOrn was determined by comparing the retention time with those for the authentic *erythro*- and *threo*-βHOrn (91.0 and 90.4 min, respectively). The retention time of the βHOrn in the degradation products of 1 was found to be 91.0 min. The absolute stereochemistry of βHOrn was elucidated by the chiral HPLC analysis (Chiralpak WH, Daicel Chemical, 4.6 × 250 mm) at a flow rate of 1.0 mL/min with 1.0 mmol/L of $CuSO_4$ aqueous solution and detected at 254 nm. The retention times of authentic L- and D-*erythro*-βHOrn were 22 and 10 min, respectively. The retention time of the βHOrn in degradation products of 1 was found to be 22 min.

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Supplementary Material Available: ^1H NMR, ^{13}C NMR, ^1H–^1H COSY, HMQC, HMBC, ROESY, UV, IR, FABMS, and HRFABMS spectra of 1 and 2 and ^1H NMR, ^{13}C NMR, ROESY, EIMS, and HREIMS spectra of 3 (25 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.

A Practical, Highly Enantioselective Synthesis of the Taxol Side Chain via Asymmetric Catalysis

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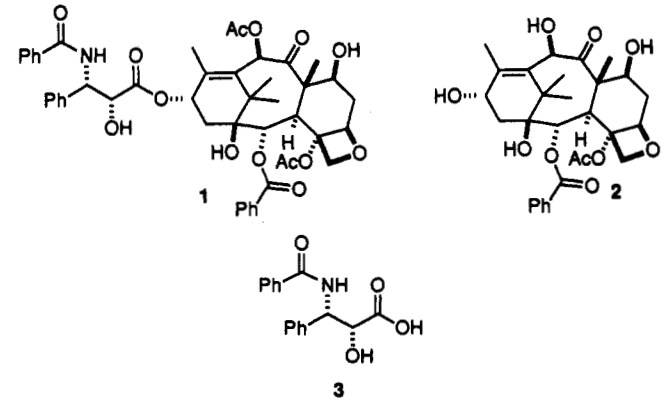
The importance of taxol's C-13 side chain to the drug's powerful antileukemic and tumor-inhibiting activity was noted in the very earliest biological studies on this remarkable molecule.[1] Side chain structure–activity relationship studies have subsequently led to other promising drug candidates[2] and to the suggestion that the 3′-amide substituent plays an important role in the preorganization of taxol for binding to microtubules.[3] Interest in synthetic routes to the *N*-benzoyl-3-phenylisoserine side chain **3** has



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