an additional 90 min. The reactor/trap assembly was then arranged in the evaporation configuration and attached to a vacuum manifold. After three freeze-thaw cycles, the volatiles were bulb-to-bulb distilled into the collection trap with magnetic stirring of the product material in the reaction trap. The residue in the reaction trap was carefully removed, and the procedure was repeated with proportionately less methyl diazoacetate. At the end of five such cycles, a total of 1.83 g of material containing the desired product was in hand. This material was subjected to rotary evaporation with trapping of the volatiles. The trapped volatiles were pooled with the unconverted hexalabled benzene for recovery. The crude product was filtered through a 1 × 8 cm column of Bio-Sil A, 100-200 mesh, with 1:1 pentane-methylene chloride elution. Evaporation gave 1.229 g of material. This material was subjected to distillation in a Kugelrohr oven (100-115 °C at 2 Torr) to yield two fractions, 0.668 g and 0.309 g. NMR indicated that the first fraction contained 90% and the second fraction 25% of the desired methyl cycloheptatriene-7-carboxylate-*1,2,3,4,5,6*-^{13}C, 0.68 g (72% yield based on recovered benzene).[21] A second preparation conducted with 2.13 g of hexalabeled benzene and 1.50 g (15.0 mmol) of methyl diazoacetate in six cycles afforded 1.34 g (8.6 mmol) of labeled methyl cycloheptatriene-7-carboxylate (70% based on recovered benzene). ^1H NMR: δ 2.56 (m, 1 H), 3.79 (s, 3 H) 5.45 (dm, 2 H), 6.26 (dm, 2 H), 6.85 (dm, 2 H). ^{13}C NMR: δ 116.9 (d), 125.6 (m), 130.6 (m).

Trimethyl Tricyclo[3.2.2.0^{2,4}]-6,8-nonadiene-3,6,7-tricarboxylate-*3,carboxyl*-$^{13}C_2$. A 5-mm NMR tube was charged with a carbon tetrachloride (0.611 g) solution of distilled methyl cycloheptatriene-7-carboxylate-7,*carboxyl*-$^{13}C_2$ (0.669 g, 4.45 mmol) and freshly distilled dimethyl acetylenedicarboxylate (1.11 g 7.83 mmol).[22] The tube was cooled in dry ice and sealed with a torch, and an initial NMR spectrum was recorded. The tube was then inserted into a $^3/_8$ in. stainless steel tube closed on one end, and this assembly was heated at 98 °C in a vertical Kugelrohr oven for 24 h. A spectrum recorded at this time indicated complete conversion to products. The contents were transferred to a 10-mL flask, and the volatiles were removed by rotary evaporation followed by Kugelrohr distillation at 110-115 °C at 2 Torr to remove the excess dimethyl acetylenedicarboxylate. The amber residue (1.28 g, 99%) solidified, mp 60-65 °C. Recrystallization from ether-pentane gave a sample with mp 75-77 °C (lit. mp 76 °C, 74-75 °C, 48-50 °C).[8-10] ^1H NMR: δ 1.76 (m, $^1J_{CH}$ = 171 Hz, 1 H), 2.06 (m, 2 H), 3.61 (d, $^3J_{CH}$ = 4 Hz), 3.78 (s, 6 H), 4.23 (m, 2 H), 6.15 (m, 2 H). ^{13}C NMR: δ 30.1 (d, $^1J_{CC}$ = 74 Hz), 171.1 (d).

Trimethyl Tricyclo[3.2.2.0^{2,4}]-6,8-nonadiene-3,6,7-tricarboxylate-*1,2,4,5,8,9*-$^{13}C_6$. The procedure described for the doubly labeled cycloheptatriene derivative was used. A mixture of carbon tetrachloride (0.426 g), hexalabeled methyl cycloheptatriene-7-carboxylate (0.68 g, 4.3 mmol), and freshly distilled dimethyl acetylenedicarboxylate (1.044 g, 7.35 mmol) afforded 1.04 g, 81%, of the Diels-Alder adduct, mp 74-75 °C, after recrystallization from pentane-ether. ^1H NMr: δ 1.77 (t, 1 H), 2.07 (dm, $^1J_{CH}$ = 177 Hz, 2 H), 3.62 (s, 3 H), 3.79 (s, 6 H), 4.23 (dm, $^1J_{CH}$ = 147 Hz), 6.15 (dm, $^1J_{CH}$ = 176 Hz). ^{13}C NMR: δ 26.8 (m), 40.1 (m), 130.8 (m).

Methyl Cyclopropene-3-carboxylate-*3,carboxyl*-$^{13}C_2$. A number of trial vacuum pyrolyses with unlabeled Diels-Alder adduct indicated that the cycloreversion reaction was best conducted in our apparatus at 410 ± 20 °C.[23] A packed quartz pyrolysis tube (300 × 15 mm) equipped with standard taper 14/20 joints and two traps fabricated from 15-mL centrifuge tubes were attached to a vacuum manifold. The pyrolysis tube was heated with two 100-mm combustion tube heaters, and the thermocouple monitor was mounted at the point of contact between the two heaters. Heating tape was used to heat the exposed portion of the pyrolysis tube and trap side arm to minimize condensation of pyrolysate. The system was thoroughly baked out under vacuum and brought to atmospheric pressure with dry helium. A 5-mL flask containing the doubly labeled Diels-Alder adduct (0.520 g, 1.73 mmol) topped with a small plug of oven-dried glass wool was attached to the pyrolysis tube, and the system was evacuated to 10^{-2} Torr. With the tube heaters at temperature (390-430 °C through regions of the pyrolysis tube) and the traps cooled with liquid nitrogen, the flask was heated to 275 °C with a small heating mantle. After a 1-h period all the material had passed through the pyrolysis column. When the system had cooled, the apparatus was brought to atmospheric pressure with helium. The pyrolysis tube was removed, and the inlet to the first trap was sealed with a small flask. The first trap was then warmed to room temperature, and the methyl cyclopropane-3-carboxylate was transferred to the liquid nitrogen cooled second trap by bulb-to-bulb distillation at 10^{-2} Torr. Magnetic stirring of the sample in the first trap facilitated this transfer. In this manner, 82 mg of methyl cyclopropene-3-carboxylate-*3,carboxyl*-$^{13}C_2$ (49%, 73.5% based on recovered starting material) was isolated. A second pyrolysis of 0.500 g of the labeled Diels-Alder adduct afforded 0.137 g (81.5%) in one pass. ^1H NMR (CD_2Cl_2): δ 2.17 (ddt, $^1J_{CH}$ = 178 Hz, $^2J_{CH}$ = 9.25 Hz, $^3J_{HH}$ = 1 Hz, 1 H), 3.63 (d, $^3J_{CH}$ = 4 Hz, 3 H), 7.08 (m, 2 H). ^{13}C NMR (CD_2Cl_2): δ 17.3 (ddtq, $^1J_{CC}$ = 77 Hz), 177.6 (ddqt).[24]

Methyl Cyclopropene-3-carboxylate-*1,2*-$^{13}C_2$. A sample of the hexalabled Diels Alder adduct (0.389 g, 1.30 mmol) was pyrolyzed in the manner previously described to yield 70 mg (72%, based on recovered starting material, purity >97%). ^1H NMR (CCl_2D_2): δ 2.17 (m, $^2J_{CH}$ = 1.4 Hz, $^3J_{HH}$ = 1.45, 1 H), 3.65 (s, 3 H), 6.93 (part of AA'MXX' pattern, $^1J_{CH}$ = 239.5 Hz, $^1J_{CC}$ = 67.3 Hz, $^2J_{CH}$ = 7.7 Hz, $^3J_{HH}$ = 1.45 Hz, $^3J_{HH}$ = 0.66 Hz, 2 H). ^{13}C NMR (CCl_2D_2): δ 104 (part of AA'MXX' pattern).

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(21) NMR indicated that the contaminants in the fractions were dimethyl maleate and dimethyl fumarate, by-products previously observed in these rhodium catalysed reactions; see reference 20.

(22) Attempts to use unpurified samples of methyl cycloheptatriene-7-carboxylate, which contained the rhodium catalyst, led to the formation of significant quantities of cyclotrimerized dimethyl acetylenedicarboxylate. While this product could be separated by chromatography, it proved to be more efficient to purify the cycloheptatriene derivative before conducting the cycloaddition.

(23) This temperature was determined by direct measurement of the reaction zone of the pyrolysis column under simulated conditions. The external thermocouple monitor used in pyrolysis was calibrated with respect to these measured temperatures.

(24) Proton coupled ^{13}C NMR of unlabeled samples of 1 showed: δ 17.3 (dtq, $^1J_{CH}$ = 178.5 Hz, $^2J_{CH}$ = 1.84 Hz, $^4J_{CH}$ = 0.4 Hz), 104.0 (ddd, $^1J_{CH}$ = 239.5 Hz, $^2J_{CH}$ = 7.7 Hz, $^2J_{CH}$ = 1.4 Hz), 177.6 (dqt, $^2J_{CH}$ = 9.25 Hz, $^3J_{CH}$ = 3.7 Hz, $^3J_{CH}$ = 1.4 Hz).

Amphidinolide E, a Novel Antileukemic 19-Membered Macrolide from the Cultured Symbiotic Dinoflagellate *Amphidinium* sp.

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Marine microorganisms are of considerable current interest as new promising sources of bioactive substances,[2] and recently several unique secondary metabolites have

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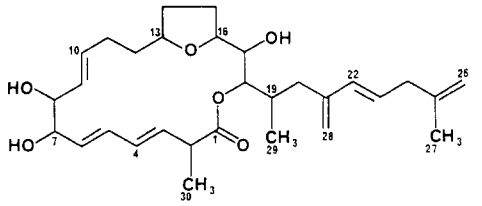
Table I. ^1H NMR Spectral Data of Amphidinolide E (1) in $CDCl_3$

position	δ	J, Hz	position	δ	J, Hz
1			16	3.56 (dt)	7.5, 7.1
2	3.26 (dq)	10.0, 6.8	17	3.72 (dd)	7.5, 4.5
3	5.59 (dd)	14.0, 10.0	18	4.66 (d)	8.3
4	6.20 (dd)	14.0, 10.6	19	2.25 (m)	
5	6.16 (dd)	14.5, 10.6	20a	2.40 (d)	13.4
6	5.53 (dd)	14.5, 8.5	20b	1.79 (m)	
7	3.88 (t)	8.5	21		
8	3.95 (t)	8.5	22	6.05 (d)	15.9
9	5.27 (dd)	15.6, 8.5	23	5.71 (dt)	15.9, 6.8
10	5.64 (m)		24 (2 H)	2.78 (br d)	6.8
11a	2.23 (m)		25		
11b	1.82 (m)		26a	4.75 (s)	
12a	1.76 (m)		26b	4.71 (s)	
12b	1.48 (m)		27 (3 H)	1.72 (s)	
13	3.41 (m)		28a	4.98 (s)	
14a	1.40 (m)		28b	4.87 (s)	
14b	1.25 (m)		29 (3 H)	0.92 (d)	6.6
15a	1.58 (m)		30 (3 H)	1.25 (d)	6.8
15b	1.33 (m)				

been isolated from marine bacteria,[3] fungi,[4] cyanophytes,[5] cryptophytes,[6] and dinoflagellates.[7] During the course of our studies on bioactive substances from Okinawan marine organisms,[8] we have investigated symbiotic microalgae associated with marine invertebrates and previously isolated several new bioactive compounds from the laboratory-cultured dinoflagellates[9,10] and haptophytes.[11] In order to obtain more biologically useful substances from symbiotic microalgae, we investigated a dinoflagellate of the genus *Amphidinium* (this is a different species of *Amphidinium* from that reported previously[9]) and now isolated a new antileukemic 19-membered macrolide, amphidinolide E (1). This paper describes the isolation and structure elucidation of amphidinolide E (1)

The dinoflagellate *Amphidinium* sp. was isolated from the Okinawan flatworm *Amphiscolops* sp. and grown unialgally at 25 °C for 2 weeks in a sea water medium enriched with ES supplement.[9c] The harvested cells were extracted with methanol/toluene (3:1), and the extracts



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were partitioned between toluene and water. The toluene-soluble fraction was subjected to silica gel column chromatography with methanol/chloroform followed by repeated reverse-phase HPLC on ODS with 88% methanol to give amphidinolide E (1, 0.9 mg) along with previously reported amphidinolide A[9a] (ca. 1 mg).

Amphidinolide E (1) was obtained as a colorless amorphous solid. The IR spectra suggested the presence of hydroxyl(s) (3450 cm^{-1}) and lactone (1735 cm^{-1}) groups, and the UV absorption maximum at 230 nm was indicative of the presence of diene chromophore. In the positive-ion FABMS, 1 showed the quasi-molecular ion peaks at m/z 501 [$(M + H)^+$, glycerol matrix] or 606 [$(M$ + diethanolamine + $H)^+$, diethanolamine matrix]. In combination with the ^1H (Table I) and ^{13}C NMR data (particularly DEPT results), the molecular formula of 1 was deduced to be $C_{30}H_{44}O_6$. The ^{13}C NMR spectrum revealed all 30 carbons, of which 13 are sp^2 carbons. Since these sp^2 carbons accounted for seven of the nine unsaturations present in the molecule, 1 was assumed to contain two rings. Analysis of the ^1H and DEPT spectra also provided evidence for the presence of six aliphatic methylenes, six oxygenated methines, two unoxygenated methines, and three methyl groups. The information obtained from the ^1H–^1H COSY spectrum easily led to the connectivities for three partial structures of C-2–C-14, C-15–C-19, and C-20–C-27.[12] Although the connectivities for C-14/C-15 and C-19/C-20 were not so clear in the COSY spectrum due to broad unresolvable patterns of multiplet proton signals heavily overlapped with each other, the relayed COSY (RCT)[13,14] spectrum showed relayed connectivity between H_3-29 (Me on C-19, 0.92 ppm) and H-20b (1.79 ppm), providing evidence for the connection between C-19 and C-20. The low-field resonance of the H-2 (3.26 ppm) suggested that this proton was located between carbonyl and olefinic carbons,[15] implying that C-2 was adjacent to the lactone carbonyl (C-1). Thus the remaining carbon, C-14, and C-15, must be connected. The chemical shifts of the protons attached to those carbons (1.40, 1.25 and 1.58, 1.33 ppm) would not permit formulation of either of these carbons as α to a carbonyl group. The geometry of the four disubstituted double bonds was determined as all *E* on the basis of the proton–proton coupling constants.[16] Of the six oxymethine protons, the H-18 resonated particularly in the low-field (4.66 ppm). This fact suggested that the ester oxygen on C-1 is connected to C-18 to construct a 19-membered macrocyclic lactone ring. Since 1

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(12) The ^1H–^1H COSY spectrum clearly showed the following correlations (H/H): 30/2, 2/3, 3/4, 4/5, 5/6, 6/7, 7/8, 8/9, 9/10, 10/11a, 10/11b, 11a/11b, 11a/12a, 12a/13, 12b/13, 13/14a, 13/14b, 15a/16, 15b/16, 16/17, 17/18, 18/19, 19/29, 20a/20b, 20a/28b, 28a/22, 22/23, 22/24, 23/24, 24/26b, 24/27, 26a/27, and 26b/27.

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(14) The RCT spectrum afforded the following relayed connectivities (H/H): 30/3, 2/4, 3/5, 4/6, 5/7, 6/8, 7/9, 8/10, 10/12a, 10/12b, 15b/17, 16/18, 18/29, and 29/20b.

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(16) $J_{3,4}$ = 14.0 Hz, $J_{5,6}$ = 14.5 Hz, $J_{9,10}$ = 15.6 Hz, and $J_{22,23}$ = 15.9 Hz: For determining the coupling constants, *J*-resolved 2D NMR was particularly useful.

was suggested to contain two rings, another ring must be formed by an ether linkage. The ^1H and ^{13}C chemical shifts of oxymethine groups (C-7, -8, -13, -16, and -17) indicated the absence of the epoxide ring.[9a,b] Since the $\Delta^{9,10}$-double bond was *E*, ether formation from C-7 or C-8 to C-13, C-16, or C-17 is unlikely. The oxygen function at C-17 is a hydroxyl group, because the H-17 signal was coupled with a hydroxyl proton by 4.5 Hz. Thus the ether linkage had to be constructed between C-13 and C-16 by process of elimination to form a tetrahydrofuran ring that is entropically most preferred. Thus the structure of amphidinolide E was concluded to be **1**.[17]

Amphidinolide E (**1**) is a novel antileukemic 19-membered macrolide with an alkyl side chain, and the substitution patterns are different from those of amphidinolides A–D obtained from a different species of the dinoflagellate *Amphidinium* sp.[2,9] Amphidinolide E (**1**) exhibited cytotoxic activity against L1210 (IC_{50} 2.0 μg/mL) and L5178Y (IC_{50} 4.8 μg/mL) murine leukemia cells in vitro.

Experimental Section

General Methods. UV and IR spectra were measured on a Beckmann DU-68 and JASCO IR-810 spectrophotometer, respectively. ^1H and ^{13}C NMR spectra were recorded on a JEOL GSX-270 spectrometer in $CDCl_3$. The 7.26 ppm resonance of residual $CHCl_3$ and 77.0 ppm of $CDCl_3$ were used as internal references, respectively. Mass spectra were obtained on a JEOL HX-100 spectrometer.

Isolation. The procedure for the algal cultivation has been previously described.[9c] The harvested cells (260 g) from 718 L of culture were extracted with methanol/toluene (3:1, 700 mL × 1 and 500 mL × 2). After addition of 1 M NaCl (0.85 L), the mixture was extracted with toluene (500 mL × 3). The toluene-soluble fraction was evaporated under reduced pressure to give a crude residue (13.8 g), which was subjected to silica gel column chromatography (Merck, silica gel 60 (7734); 2.7 × 47 cm) eluted with methanol/chloroform (5:95). The fraction eluting from 320 to 420 mL was further separated by the second silica gel column chromatography (1.6 × 28 cm) eluted with methanol/chloroform (4:96). The fraction eluting from 60 to 160 mL was then purified by HPLC (YMC-Pack AM-323 S-5 120A ODS, Yamamura Chemical, 10 × 250 mm; flow rate, 2.5 mL/min; UV detection at 254 nm; eluant, 88% methanol) to afford amphidinolide E (**1**, 0.9 mg, t_R 12.2 min) together with amphidinolide A (ca. 1 mg, t_R 12.8 min).

Amphidinolide E (1): colorless amorphous solid; UV (MeOH) 230 nm (ε 26 000); IR (film) 3450, 1735, 1460, 1170, and 990 cm^{-1}; ^1H NMR (Table I); ^{13}C NMR ($CDCl_3$) (sp^2 carbons) 174.42 s (C-1), 144.68 s, 144.00 s (C-21 and 25), 135.14 d, 134.93 d, 134.15 d, 133.34 d, 131.40 d, 131.37 d, 129.41 d, 127.93 d (C-3, 4, 5, 6, 9, 10, 22, and 23), 115.70 t, 110.71 t (C-26 and 28); (oxygenated methines) 79.86 d, 78.27 d, 78.04 d, 77.58 d, 76.68 d, 73.20 d (C-7, 8, 13, 16, 17, and 18); (unoxygenated methines) 44.06 d, 32.34 d (C-2 and 19); (aliphatic methylenes) 41.26 t, 36.07 t, 32.60 t, 29.94 t, 28.95 t, 27.14 t (C-11, 12, 14, 15, 20, and 24); (methyls) 22.53 q, 17.52 q, and 15.36 q (C-27, 29, and 30); FABMS (positive ion, glycerol matrix) *m/z* 501 $(M + H)^+$ and 483 $(M - H_2O + H)^+$; FABMS (positive ion, diethanolamine (DEA) matrix) *m/z* 606 $(M + DEA + H)^+$ and 588 $(M + DEA - H_2O + H)^+$.

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Supplementary Material Available: COSY, RCT, *J*-resolved 2D NMR, and ^{13}C and DEPT spectra of Amphidinolide E (4 pages). Ordering information is given on any current masthead page.

(17) The stereochemistry of all chiral centers in **1** remains undefined.

Preparation of α-Fluoro Carboxylic Acids and Derivatives

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Incorporation of fluorine in organic molecules leads to changes in chemical and physical properties that yield products which have applications in many fields. Of particular interest is the ability of fluorine to modify the reactivity of biologically active molecules.[1] α-Fluoro carbonyl derivatives[2] are of importance in themselves and can also be used as building blocks to synthesize more complex molecules.[3] α-Fluoro carboxylate esters are often prepared by condensation reactions of the extremely toxic fluoroacetates.[4] They have also been synthesized from bromofluoroacetates.[5] The advantage of the method presented here is that the carbon skeleton is in place prior to introduction of the fluorine atom.

The fluorination of silyl enol ethers in $FCCl_3$ at −78 °C with fluorine diluted in nitrogen in the formation of α-fluoro ketones and α-fluoro aldehydes has previously been reported.[6] In this note, we report that we have been able to extend this methodology to the synthesis of α-fluoro carboxylic acid and derivatives. Esters,[7] malonates,[8] carboxylic acids,[8] dimethyl amides,[9] and a lactone[7] were silylated with chlorotrimethylsilane by standard procedures. The results of the fluorination of these compounds are presented in Table I.

Several observations are worth noting. The difluorocarbonyl compound was the major byproduct formed in the reaction of the ketene silyl acetals. It generally accounted for lower yields of those silyl derivatives with an α-hydrogen: compare entries 1–3 with entry 4 and entry 5 with entries 6 and 7. The overfluorinated impurity could be eliminated during purification by chromatography. Attempts to minimize the formation of difluoro compounds were unsuccessful.

Of all the silyl derivatives investigated, the yields for the free carboxylic acids were the highest. For these compounds no difluorinated product was obtained. Presumably, the fact that F_2 can interact equally with either silyl group on the ketene bis(trimethylsilyl) acetal is the basis of this result. The α-fluoro carboxylic acids could also be easily prepared by hydrolysis and decarboxylation of the corresponding malonates.[10] Thus, α-fluorophenylacetic

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