it has two spirofused six-membered ketal ring systems in place of the more commonly encountered spiroketal involving six- and fixe-membered rings. The presence of two fused tetrahydropyran rings (D, E) is also unique.

Acanthifolicin exhibits ED_{50}'s of 2.8×10^{-4}, 2.1×10^{-3}, and 3.9×10^{-3} mcg/mL, respectively, against P388, KB, and L1210 cell lines. In vivo antitumor tests and other biological activity evaluation are in progress.

Efforts are under way to isolate microorganisms associated with *P. acanthifolium* that may produce acanthifolicin. In this connection it may be noted that a macrolide polyether antibiotic, aplasmomycin, has been isolated from a marine bacterium.[10]

Acknowledgment. This investigation was supported by Grant CA-17256, CA-17562, and Contract N01-CM-67108 awarded by the National Cancer Institute, DHEW. We thank Ms. Lin Craft for sponge identification and assistance in sample collection. 360-MHz ^1H NMR spectra were obtained at the Purdue Biochemical Magnetic Resonance Laboratory supported by NIH Grant RR 01077.

Supplementary Material Available: A listing of the final parameters, bond angles, torsion angles, structure factors, bond lengths, and Bijvoet differences is available (40 pages). Ordering information is given on any current masthead page.

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Okadaic Acid, a Cytotoxic Polyether from Two Marine Sponges of the Genus *Halichondria*

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A new polyether derivative of a C_{38} fatty acid, okadaic acid (**1**), has been isolated independently from two sponges, *Halichondria* (syn *Reniera*) *okadai* Kadota, a black sponge, commonly found along the Pacific coast of Japan,[1] and *H. melanodocia*, a Caribbean sponge collected in the Florida Keys.

Mammalian toxicity of a crude extract of *H. okadai* guided the isolation of a colorless crystalline solid ($\sim 10^{-4}$% from wet animal). The methanolic homogenate was further extracted at room temperature thrice with methanol and once with acetone. After partial solvent evaporation, the 70% aqueous residue was washed with *n*-hexane. The aqueous residue after complete organic solvent removal was finally extracted with ethyl acetate. Repeated chromatography of the organic residue [polystyrene gel (Hitachi 3019), MeOH; Sephadex LH-20, MeOH; silicic acid, *n*-hexane/acetone 5:1], followed by crystallization from MeOH and recrystallization from dichloromethane/hexane, furnished the acid, mp 171-175 °C, $[\alpha]^{20}_D$ +21° (*c* 0.33, $CHCl_3$). Okadaic acid was toxic (LC_{50} 192 μg/kg; ip mice) and inhibited growth of KB cells by more than 30% at 2.5 ng/mL and more than 80% at 5 ng/mL.

From *H. melanodocia* the Oklahoma group isolated okadaic acid by a procedure similar to that used for acanthifolicin.[2] A concentrated 2-propanol extract of the sponge collected near Summerland Key, FL, was diluted with water and extracted continuously with CH_2Cl_2. The CH_2Cl_2 solubles were suspended in 10% aqueous MeOH and extracted successively with hexane, CCl_4, and $CHCl_3$, as the water content of the aqueous MeOH phase was increased after the hexane and CCl_4 washes to 20 and 30%, respectively. The $CHCl_3$ solubles were chromatographed over Sephadex LH-20 (MeOH-$CHCl_3$, 1:1), and fractions exhibiting mouse toxicity were chromatographed over deactivated[3] silica gel ($CHCl_3 \rightarrow CHCl_3$-5% MeOH) to give the toxic component as a brown powder. One crystallization from benzene, followed by several recrystallizations from benzene-$CHCl_3$, afforded a white crystalline solid, mp 164-166 °C, $[\alpha]^{25}_D$ +25.4° (*c* 0.24, $CHCl_3$); approximate yield, 1×10^{-4}% of wet sponge weight.[4] The pure compound exhibited ED_{50} values[5] of 1.7×10^{-3} and 1.7×10^{-2}, respectively, against P388 and L 1210 cell lines. Okadaic acid was toxic at doses of ≥0.12 mg/kg (ip) and showed no tumor inhibition at subtoxic doses when tested in vivo against P388 lymphocytic leukemia.

^1H NMR spectra (360, 270 MHz) of the two samples of okadaic acid were identical. Nearly complete assignments are shown in Chart I.[6] UV (end absorption) and IR (3450, 1740, 1080, 880 cm^{-1}) spectra were rather uninformative. An electron impact mass spectrum exhibited its highest mass peak at *m/z* 786 for a composition of $C_{44}H_{66}O_{12}$. Only a trivial fragmentation peak at *m/z* 771 was readily interpretable.[7] ^{13}C NMR spectra in $CDCl_3$ and pyridine-d_5 revealed 44 peaks: 1 carboxyl singlet at 179.3 ppm, 6 olefinic carbons [exo-methylene at 147.9, 111.5 ppm; trisubstituted olefin (137.7, 126.8 ppm); trans disubstituted olefin, 135.5, 131.6 ppm], 3 ketal or hemiketal singlets near 100 ppm, 12 carbons bearing oxygen between 85.9 and 60.4 ppm (one quaternary carbon at 75.2, one CH_2 at 60.4 ppm, all other methines); the remaining 22 highfield signals (46.0-11.0 ppm) included 5 methyls and 3 methines.[8] The carbon data and preparation of a tetraacetate (vide infra) showed that okadaic acid possesses 13 (1 C=O, 12 C-O) rather than 12 oxygens, as had been indicated by EI mass spectrometry. A field desorption mass spectrum of *p*-bromophenacyl okadaate, mp 134-135 °C, subsequently confirmed a molecular formula of $C_{44}H_{68}O_{13}$.

Treatment of **1** with diazomethane furnished methyl okadaate, mp 127-133 °C (hexane-benzene); $[\alpha]^{25}_D$ +28° (*c* 0.38, $CHCl_3$); IR ($CHCl_3$) 3600, 3500 (brd), 1745, 1725 cm^{-1}; ^1H NMR signals in ref 9.

Preparation of an amorphous tetracetate (Ac_2O, pyridine, 20 h, room temperature), $[\alpha]^{20}_D$ +53° (*c* 1.4, $CDCl_3$), unambiguously proved presence of four hydroxyls by four acetate singlets at δ

† Suntory Institute for Bioorganic Research, Osaka, 618, Japan.

(1) A preliminary account of this work was presented at the Third International Symposium on Marine Natural Products in Brussels, Belgium, Sept 16-19, 1980. In part from the Ph.D. Dissertation of K.T., University of Hawaii, 1980.

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(3) Silica gel was slurried in methanol containing 5% water, packed, and then flushed with $CHCl_3$ before initiating the chromatography.

(4) Initially designated melanodocin in U.S. Patent Application SN 170927.

(5) See ref 2.

(6) Measured in $CDCl_3$ on an NT-360 instrument at the Suntory Institute for Bioorganic Research, Osaka, Japan.

(7) After the structure **1** was known, the following major peaks were interpretable from a FDMS of the *p*-bromophenacyl ester: *m/z* 519, fission of C-12,13; *m/z* 241, fission of C-26,27; and *m/z* 101, fission shown in diagram.

(8) The crowded high field region did not permit unambiguous distinction between singlet and triplet signals among the remaining 14 carbons.

(9) Methyl okadaate: ^1H NMR (270 MHz, $CDCl_3$) [chemical shift-(multiplet, *J*, (Hz), partial assignments)] 0.93 (d, 6.75), 1.04 (d, 6.75, H-42), 1.07 (d, 6.75), 1.37 (s, H-44), 1.73 (s, H-43), 3.25-3.75 (7 H, m), 3.81 (s, OCH_3), 3.90-4.20 (4 H, m), 4.48 (q, 8, H-16), 5.06 (s, H-41), 5.48 (dd, 15.5, 8, H-15), 5.58 (dd, 15.5, 8, H-14) ppm; exchangeable absorptions at 2.52, 2.89, and 4.94 ppm. Besides these absorptions the spectrum contained complex overlapping multiplets in the range 1.20-2.35 ppm.

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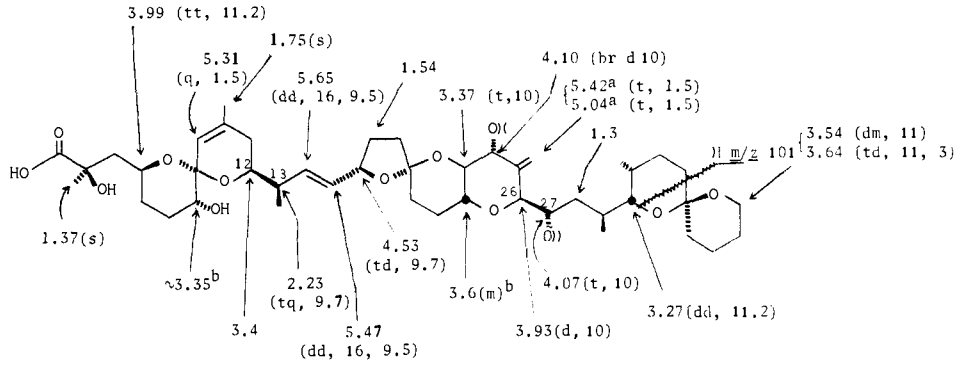
Chart I[a]



[a] a and b indicate assignments interchangeable.[3]

2.15, 2.07, 2.03, and 1.94 in its ^{1}H NMR spectrum.[10]

Characterization of **1** suggested to the Oklahoma group that acanthifolicin[2] was an episulfide of okadaic acid. This was proven by refluxing acanthifolicin methyl ester in absolute EtOH with Zn–Cu couple[11] for 6 h to effect desulfurization. ^{1}H NMR (270 MHz) analysis indicated that the reaction product consisted of a 60:40 mixture of methyl okadaate and starting material. Chromatography over 10% silver nitrate impregnated silica gel (5% MeOH in $CHCl_3$) gave methyl okadaate, identical with the natural product methyl ester by mp, mmp, optical rotation, IR spectra, and 270-MHz ^{1}H NMR spectra. In a separate experiment methyl okadaate was found to be stable to the Zn–Cu reaction conditions. Because of the known absolute stereochemistry of acanthifolicin,[2] these experiments also establish the absolute stereochemistry of okadaic acid: 2*R*,4*S*,7*R*,8*R*,12*S*,13*R*,16*R*,19*S*,22*R*,23*S*,24*R*,26*S*,27*S*,29*S*,30*S*,31*R*,34*S*. The C-14 double bond has *E* configuration.

Attempts by the Hawaii group to crystallize Rb and Tl(I) okadaate or tribenzoyl methyl okadaate failed, as did attempts at Cornell to solve the total molecular structure by diffraction studies of methyl okadaate or *p*-bromophenacyl okadaate. An *o*-bromobenzyl okadaate was prepared by refluxing triethylammonium okadaate and an excess of *o*-bromobenzyl bromide in acetone for 36 h. Purification on silicic acid and crystallization (2×) from CH_2Cl_2–hexane furnished thin plates, mp 196–197 °C. The ^{1}H NMR spectrum proved that no structural changes had taken place during esterification.

Crystals of the *o*-bromobenzyl ester of okadaic acid were suitable for an X-ray diffraction experiment. Preliminary X-ray photographs showed monoclinic symmetry and accurate lattice parameters of a = 11.572 (3), b = 10.226 (3), C = 21.547 (6) Å; β = 97.74 (2)° were obtained from a least-squares fit of 15 moderate 2θ values. Systematic extinctions and the known chirality were uniquely accommodated by the space group $P2_1$, and the density indicated one molecule of $C_{51}H_{73}BrO_{13}$ formed the asymmetric unit. All unique diffraction maxima with $2\theta \leq 100°$ were collected on a computer-controlled four-circle diffractometer using graphite monochromated Cu Kα (1.54178 Å) X-rays and a variable speed, 1° ω scan. After correction for Lorentz, polarization, and background effects, 1909 (64%) of the 2982 surveyed reflections were judged observed ($|F_o| \geq 3\sigma(F_o)$).

An initial phasing model was achieved by a combination of standard heavy atom techniques and direct methods.[12] This led to a phasing model with 52 atoms. The remaining 13 nonhydrogen atoms were located on a difference synthesis, and 72 out of the 73 hydrogens were included. Full-matrix least-squares refinement with anisotropic thermal parameters for the nonhydrogen atoms and isotropic hydrogens have converged to the current residual of 0.063. Inclusion of anomalous scattering for bromine led to a residual of 0.064 for the enantiomer. Please see the paragraph about supplementary material for further crystallographic details.

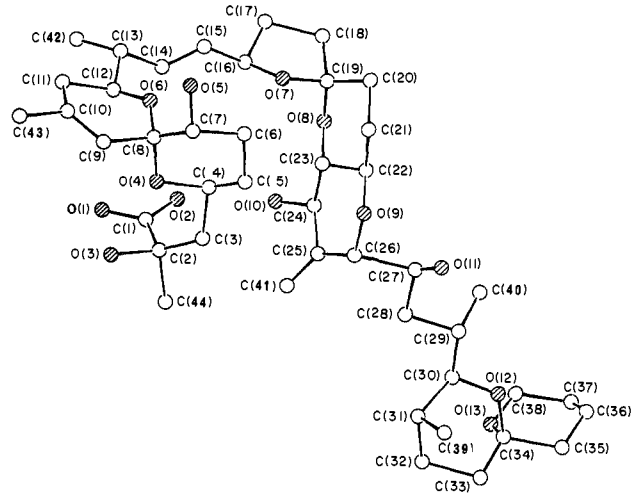


Figure 1. Computer-generated perspective drawing of the final X-ray model of okadaic acid. The *o*-bromobenzyl fragment attached to O(1) is omitted for clarity and no absolute configuration is implied.

A computer generated perspective drawing of the current X-ray model minus the *o*-bromobenzyl group is shown in Figure 1. All of the tetrahydropyran rings are in the chair conformation, and the tetrahydrofuran ring is in the envelope (C_s) conformation with O(7) as flap.

Okadaic acid is a complex derivative of a C_{38} fatty acid. Its structural features suggest that it belongs to the class of compounds known as ionophores, which hitherto had been known only from terrestrial microorganisms. It is likely that okadaic acid is a metabolite of an epiphytic microorganism, rather than of *Halichondria* spp.[16] On the other hand, our structural studies of the marine toxins palytoxin and ciguatoxin[13] strongly indicate that these two compounds possess many structural features normally associated with ionophores. The ionophoric structure of these marine toxins has been foreshadowed by their physiological properties.[14,15]

(10) FDMS: m/z 995 [100%, (M + Na)$^+$], 978 [72% (M + Na–OH)$^+$], 976 [6% (M + Na – H_2O)$^+$], 951 [66% (M + Na – CO_2)$^+$], 939 (42%), 931 (61%), 814 (46%).

(11) See Herin, M.; Tursch, B. *Bull. Soc. Chim. Belg.* **1976**, *85*, 707–719.

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(16) **Note Added in Proof**: Professor T. Yasumoto has informed P.J.S. that he has isolated okadaic acid from the dinoflagellate *Procentrum lima*. *P. lima* therefore is a likely progenitor of okadaic acid.

Acknowledgment. The work at Oklahoma was supported by Grant 04-7-158-44067 from the Office of Sea Grant, NOAA, department of Commerce. We thank Dr. R. E. Schroeder, Summerland Key, FL, and Ms. Lin Craft for assistance in collecting *H. melanodocia*, Dr. K. Ruetzler, Smithsonian Institution, for sponge identification, and Dr. P. Schmidt, Oklahoma Medical Research Foundation, Oklahoma City, OK, for use of a 270-MHz NMR spectrometer. Field desorption mass spectra were provided by the mass spectrometry facility at Massachusetts Institute of Technology, supported by a grant (principal investigator, Professor K. Biemann) from the Biotechnology Research Branch, Division of Research Resources. We gratefully acknowledge grants from the Phillips Petroleum Company, Bartlesville, OK, and the NSF (GP 38410) which aided in the purchase of NMR spectrometers. The Hawaii group thanks Drs. J. Dallas and J. Matson (UC, Davis), Dr. E. Kho (Stanford), and Mr. R. Okuda for ^{1}H NMR spectra and the National Science Foundation and the U.S. Food and Drug Administration for financial support. Crystallographic work was supported by NIH through a training grant to D.V.E. and CA 24487 to J.C.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, bond distances, and bond angles (8 pages). Ordering information is given on any current masthead page.

Aspects of Homogeneous Carbon Monoxide Fixation: Selective Conversion of Two Carbonyl Ligands on $(\eta^5\text{-}C_5H_5)Fe(CO)_3^+$ to C_2 Organic Compounds

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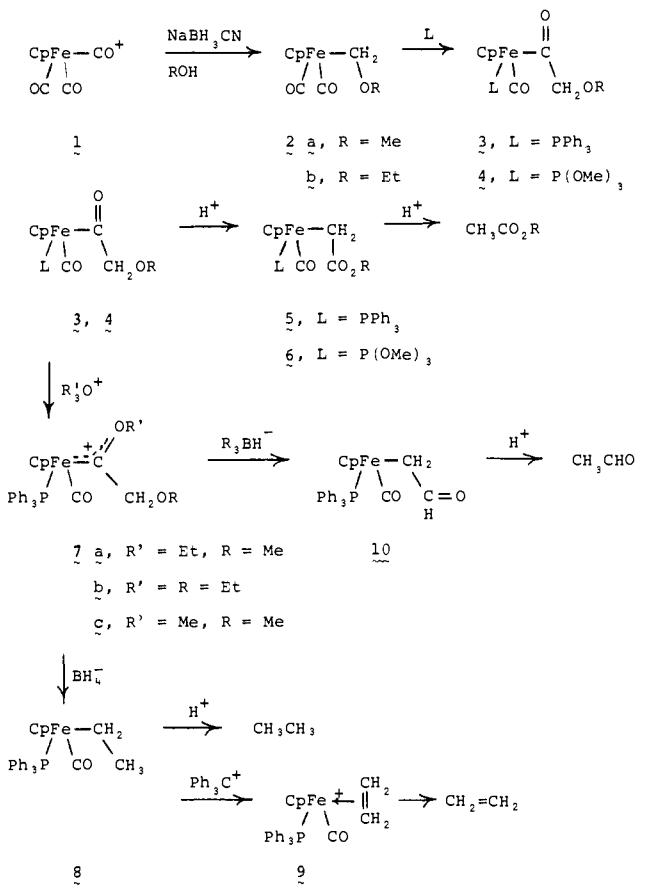
The conversion of synthesis gas—CO and H_2 mixtures derived from coal—into organic compounds will provide an alternative source of C_2–C_4 organic feedstocks heretofore obtained from petroleum.[1] Such conversions using heterogeneous catalysts in the Fischer–Tropsch synthesis and related processes are limited by the complex organic mixtures produced. Switching to homogeneous catalysts, however, should engender potentially high and manipulative product selectivity.[2] Soluble transition organometallic complexes that catalytically change synthesis gas into ethylene glycol[3a] or ethanol[3b] and stoichiometrically reform CO ligands into the C_2 compounds ethane or ethylene,[4a] acetaldehyde,[4b] methyl acetate,[4c] or a coordinated enediolate of glycolaldehyde[4d] have been reported. Rational design of homogeneous catalysts for the selective transformation of synthesis gas into organic feedstocks requires further mechanistic details on reduction of CO ligands, subsequent synthesis reactions (i.e., chain growth of the C_1 ligand), and elimination of the desired organic molecule.

We now report viable reaction pathways for the stoichiometric transformation of two CO ligands on $CpFe(CO)_3^+$ (**1**) (Cp = η^5-C_5H_5) selectively to the C_2 organic compounds ethane, ethylene, methyl (or ethyl) acetate, or acetaldehyde. The first step requires fixation of a CO ligand: 1 equiv of $NaBH_3CN$ in methanol or ethanol reduces $CpFe(CO)_3^+BF_4^-$ (**1**) to the known η^1-alkoxymethyl complexes $CpFe(CO)_2CH_2OR$ [**2a**, R = Me; **b**, R = Et].[5] After vacuum removal of solvent and $CpFe(CO)_2H$, **2a** (55%) and **2b** (33%) were isolated by column chromatography.[6] Alkoxyacetyl complexes $CpFe(CO)L(COCH_2OR)$ [**3**, L = PPh_3; **4**, L = $P(OMe)_3$; **a**, R = Me; **b**, R = Et], which are derived from **2a,b**, then serve as key intermediates in the C_2-coordinated ligand reactions (Scheme I).[7]

Scheme I



Refluxing CH_3CN solutions of **2** and 100% excess of PPh_3 or $P(OMe)_3$ for 4 and 10 days, respectively, gave the alkoxyacetyl complexes **3** and **4**. After recrystallization from CH_2Cl_2–heptane, **3** and **4** were obtained in 30–50% yields as air-stable yellow solids.[8] These vigorous reaction conditions exemplify the difficulty with which alkoxymethyl ligands undergo alkyl–acyl migratory insertion;[9] comparable treatment of $CpFe(CO)_2CH_3$ affords 80%

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