Journal of Natural Products
Vol. 58, No. 12, pp. 1801-1807, December 1995

ANTITUMOR ACTIVITY AND STEREOCHEMISTRY OF ACETYLENIC ALCOHOLS FROM THE SPONGE *CRIBROCHALINA VASCULUM*

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ABSTRACT.—Antitumor bioassay-guided fractionation of the organic extract of the marine sponge *Cribrochalina vasculum* resulted in the isolation of several closely related cytotoxic acetylenic alcohols [**1–8**], the structures of which were assigned on the basis of chemical and spectral studies. 3-Hydroxyeicos-(4*E*)-en-1-yne [**1**], 3-hydroxydocosa-(4*E*,15*Z*)-dien-1-yne [**2**], 3-hydroxy-16-methyleicos-(4*E*)-en-1-yne [**3**], 3-hydroxy-19-methyleicos-(4*E*)-en-1-yne [**4**], 3-hydroxy-21-methyldocosa-(4*E*,15*Z*)-dien-1-yne [**5**], and 3-hydroxy-14-methyldocosa-(4*E*)-en-1-yne [**6**] are enantiomers of known compounds, while 3-hydroxyheneeicos-(4*E*)-en-1-yne [**7**] and 5-hydroxy-16-methyleicos-(3*Z*)-en-1-yne [**8**] are new metabolites isolated as minor components. The absolute configuration of C-3 in **1–7** and C-5 in **8** has been assigned as *S* using the modified Mosher's method. Compounds selected from this series showed selective in vitro antitumor activity against the H-522 non-small cell lung line and the IGROV-1 ovarian line. Synthetic racemic **1** demonstrated a modest dose-related therapeutic activity in a preliminary in vivo xenograft assay based on the latter cell line.

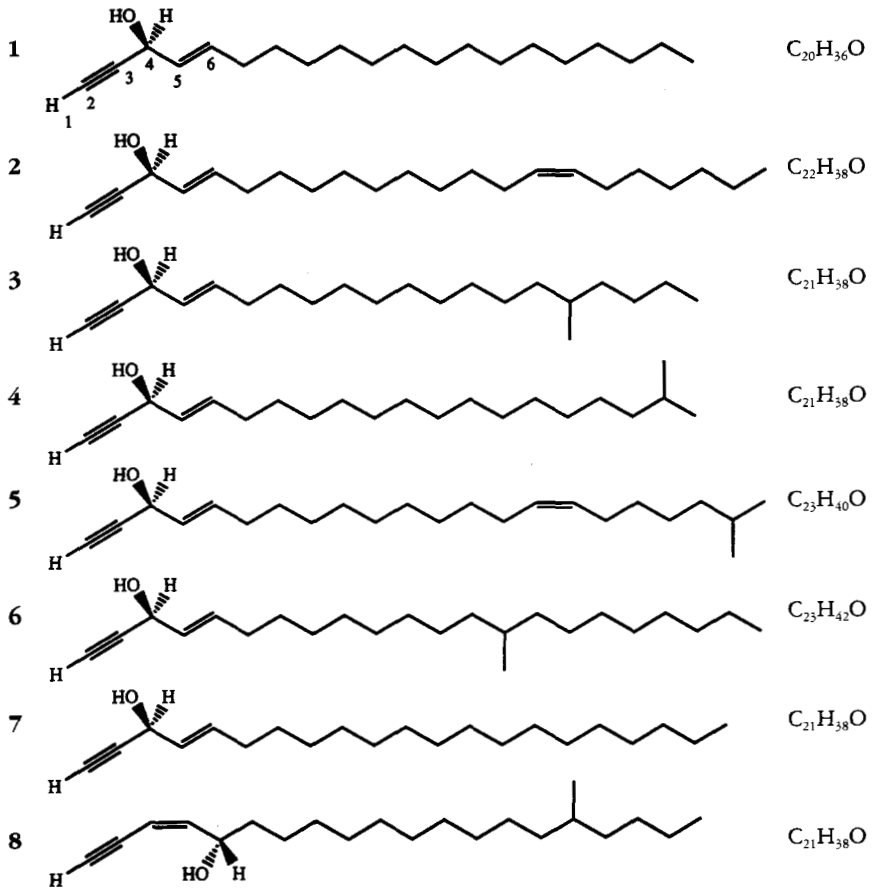
Earlier studies on the lipophilic extracts of marine sponges belonging to the genera *Petrosia* (1–5), *Xestospongia* (6), *Siphonochalina* (7,8), *Reniera* (9), and *Cribrochalina* (10) have led to the discovery of long-chain polyacetylenic alcohols with antibacterial, antifungal and cytotoxic activities. Recently, several new acetylenic alcohols have been reported in two separate studies of the Caribbean sponge *Cribrochalina vasculum* (11,12). These latter compounds have simpler structures with monoacetylene functionalities.

In our continuing search for antitumor and anti-HIV natural products from marine organisms, we found that the organic extract of *Cribrochalina vasculum* van Soest (family Niphatidae, order Haplosclerida) gave an unusual profile of differential cytotoxicity in the NCI 60-cell-line antitumor primary screen. Bioassay-guided fractionation of the lipophilic extract revealed the presence of a series of acetylenic alcohols, six of which proved to be stereoisomers of previously reported compounds (11,12). Two new acetylenic compounds, 3-hydroxyheneeicos-(4*E*)-en-1-yne [**7**] and 5-hydroxy-16-methyleicos-(3*Z*)-en-1-yne [**8**], were also isolated as minor components. We prepared Mosher ester derivatives and independently assigned the absolute stereochemistry for **1–7** at C-3 and at C-5 for **8**. We present here the purification, stereochemical analysis, and biological data for compounds **1–8**.

RESULTS AND DISCUSSION

The MeOH-CH_2Cl_2 (1:1) crude extract of *Cribrochalina vasculum* was partitioned between hexane and 90% MeOH, which concentrated the cytotoxic activity in the hexane fraction. After further separation of this fraction by vlc on a cyano-bonded phase, the major cytotoxic constituents were located in a fraction containing a series of structurally related, long-chain acetylenic alcohols similar to those reported previously (11,12). Following final purification by reversed-phase hplc, eight compounds were isolated.

Enynols **1–6** were readily identified by analysis of ^{1}H- and ^{13}C-nmr data, including DEPT, COSY, HMQC, and HMBC; compounds **1–5** had been isolated by Gunasekera



and Faircloth (11) from a Belize collection, while **2–4** and **6** were isolated by Aiello *et al.* from a Bahamas collection of the same sponge (12). Gunasekera and Faircloth assigned trans geometry to the isolated double bond in **2** on the basis of the absence of ir absorptions between 730 and 675 cm^{-1}. Although complete assignments of the ^{13}C-nmr data were hampered by the severe overlapping of signals in the aliphatic carbon regions, we were able to assign some signals, including the allylic carbons in our isolates **2** and **5**, from the corresponding HMQC and HMBC spectra. The signals of carbons allylic to the isolated double bond, which appeared at δ 27.19, 27.20, and 27.20, 27.24 in **2** and **5**, respectively, were in good agreement with a *Z* configuration at C-15 for both compounds. A lower field chemical shift value would be expected for carbons allylic to a double bond with *E* geometry (12,13). Because the spectral data of our isolate were identical to the reported values for **2** and **5**, it is, therefore, likely that the two compounds from the Belize collection should have the same *Z* configuration.

Aiello *et al.* assigned the stereochemistry at C-3 of dextrorotatory **2–4** and **6** as *R*, using the cd spectra of the corresponding *p*-bromobenzoate derivatives (12) based on an earlier report by Shim *et al.* (14) on falcarinol, a compound with a similar array of functional groups, a secondary alcohol flanked by an acetylene and an olefin. Guo *et al.* (15) recently confirmed this stereochemical assignment for **2** via the Mosher ester nmr method (16–18). Since our recent work with falcarinol and several analogues (19) revealed that the cd method used for the determination of chirality of allylic alcohols cannot be extended reliably to such enynols, as suggested by Shim *et al.* (14), we also used the modified Mosher's method (16–18) to determine the stereochemistry at C-3. The *R*-

and *S*-Mosher ester derivatives of **2**, **3**, and **6**, prepared by the usual procedure from the *S*- and *R*- acid chlorides, respectively, showed ^{1}H-nmr chemical shift differences ($\Delta\delta=\delta_S-\delta_R$, Table 1) in agreement with the *S* configuration at C-3, opposite to that assigned previously. Since the Mosher ester analysis of Guo *et al.* (15) agreed with the stereochemical assignments made by Aiello *et al.* (12) on the basis of cd data, we prepared the *p*-bromobenzoate of our **2** and recorded its cd spectrum. As illustrated in Figure 1, the cd spectrum we obtained was opposite in sign to that reported by Aiello *et al.* Thus, our data from both analyses were in agreement and supported the 3*S* configuration, indicating that our compound was the enantiomer of the compound reported from *C. vasculum* by Aiello *et al.*[1] Because our samples **1–6** have the same sign and magnitude of optical rotation, it is reasonable to assume that they should all have the *S* stereochemistry at C-3.

TABLE 1. ^{1}H-Nmr (500 MHz) Spectral Data for the MTPA Esters (δ, $CDCl_3$).

H no.	2			3			6			8		
	δ_S	δ_R	$\Delta\delta$ (Hz)	δ_S	δ_R	$\Delta\delta$ (Hz)	δ_S	δ_R	$\Delta\delta$ (Hz)	δ_S	δ_R	$\Delta\delta$ (Hz)
H1 . . .	2.606	2.567	19.5	2.607	2.564	21.5	2.605	2.567	19.3	3.235	3.225	4.9
H3 . . .										5.646	5.615	15.6
H4 . . .	5.474	5.586	−55.9	5.475	5.583	−53.5	5.474	5.584	−55.2	5.929	5.798	65.4
H5 . . .	6.003	6.053	−24.9	6.005	6.052	−23.4	6.004	6.054	−24.9			
H6 . . .	2.023	2.065	−21.0	2.024	2.063	−20.0	2.023	2.066	−21.5	1.720	1.780	−30.0
H7 . . .										1.593	1.652	−29.8

It is somewhat surprising to find enantiomers of the same compounds in different collections of the same species, although our collection was made at much greater depth than others (11,12,15). (It should be noted that the distinctive morphology of the sponge leaves little room to question the species identification.) More surprising were the results of comparing our **2** and **3** directly with Gunasekera compounds **2** and **3** from *C. vasculum* collected in Belize (11).[2] These four compounds were treated with *S*-MTPA-Cl; subsequent ^{1}H-nmr analysis revealed that our compounds gave a single diastereomeric product, while two diastereomers were derived from the Gunasekera samples. In each case, the 3*S* enantiomer was the major constituent (for Gunasekera **2**, ca. 7:3 ratio; for HBOI **3**, ca. 11:9 ratio); this would explain the disparity in optical rotations in compounds from different collections. Scalemic mixtures are known from nature (20), but are rare.

A peak eluting after compounds **1–5** in the prep. reversed-phase hplc was concentrated to give a minor component. Compound **7**, isolated as a white solid, analyzed for $C_{21}H_{38}O$ by hreims. Its ^{1}H-nmr spectrum showed one methyl triplet, indicating that this compound had a normal alkyl chain. The downfield olefinic regions showed signals similar to those in **1** and **3–5**. The absence of olefinic protons around δ 5.3 in the ^{1}H-nmr spectrum was consistent with the molecular formula and only two olefin signals (δ 129.3, 134.7) were observed in the ^{13}C-nmr spectrum. In addition, the ^{13}C-nmr, uv, and mass spectra were very similar to those of **1**, suggesting that compound **7** is a homologue

[1]Direct comparison of our **2** and Aiello's compound **1** (12), by treatment of each compound with *S*-MTPA-Cl, confirmed that the two compounds were of opposite absolute configuration. We are grateful to Prof. E. Fattorusso, Università degli Studi di Napoli "Federico II," for providing a sample of this compound for this comparison.

[2]We are grateful to Dr. S.P. Gunasekera, Harbor Branch Oceanographic Institution (HBOI), FL, for providing us with samples of his compounds **2** and **3**.

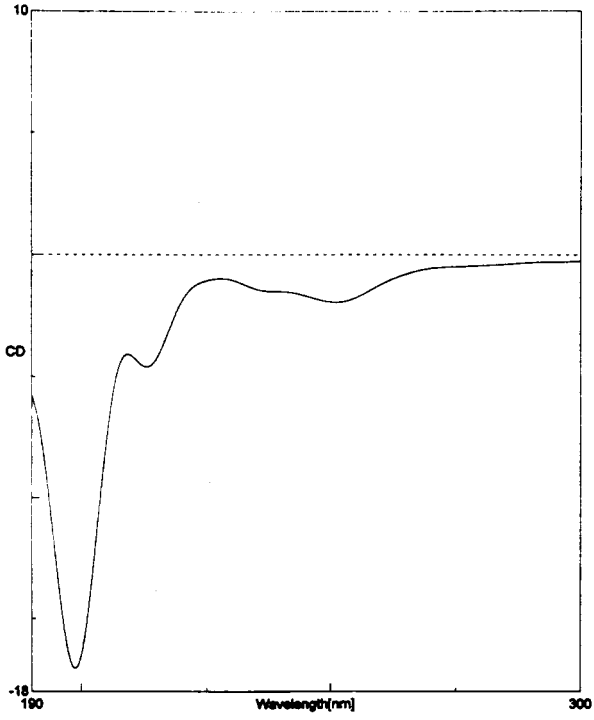


FIGURE 1. Cd spectrum of the *p*-bromobenzoate of **2** in MeOH.

of **1**, i.e., 3-hydroxyheneeicos-(4*E*)-en-1-yne. The stereochemistry at C-3 in **7** was deduced by comparison of optical rotation data with compounds **1–6**, because the paucity of available material precluded the application of Mosher's method. Inasmuch as all of these compounds gave positive optical rotations of comparable magnitude, the chirality of C-3 in **7** was assigned as *S*.

A more polar fraction which eluted earlier than **1–7** on the prep. reversed-phase hplc showed ^{1}H-nmr features different from those compounds discussed earlier. Further purification of this fraction on a semi-prep. C_{18} column yielded a colorless oil [**8**], which gave *m/z* 306.2919 by hreims, corresponding to the molecular formula $C_{21}H_{38}O$. The three degrees of unsaturation were the same as in **3**, **4**, and **7**. The downfield region of the ^{1}H-nmr spectrum showed signals with coupling patterns different from those observed for **1–7**. The carbinol proton signal which appeared around δ 4.8 in **1–7** moved upfield to δ 4.66. A doublet corresponding to a terminal acetylene functionality appeared at δ 3.11. The remaining portion of the spectrum contained a methyl doublet and a methyl triplet, indicating a branching methyl in the alkyl chain. A ^{1}H-^{1}H COSY experiment revealed a conjugated enyne system, which was further coupled to the methine signal at δ 4.66, consistent with the absence of allylic methylene resonances at 2.0 ppm in this isomer. Two-dimensional nmr (HMQC, HMBC) data provided further evidence for a conjugated enyne partial structure. The geometry of the olefin is likely to be cis since an 11.5 Hz vicinal coupling constant was observed. Additional evidence for this assignment came from the Ft-ir spectrum, in which absorptions at 758, 720, 681, and 637 cm^{-1} were observed, whereas the diagnostic absorption for trans double bonds around 975 cm^{-1} was absent. Due to overlapping signals in the aliphatic region, the site of methyl branching could not be ascertained by nmr analysis. Instead, it was deduced from an analysis of the fragmentation patterns of the eims data. Two key fragments at

m/z 249 and 221, corresponding to $[M-C_4H_9]^+$ and $[M-C_6H_{13}]^+$, respectively, and the absence of a fragment ion at *m/z* 235 securely placed the methyl group at the C-16 position.

To determine the absolute stereochemistry at C-5, Mosher (MTPA) esters were prepared, purified by vlc and analyzed by 500 MHz ^{1}H-nmr spectroscopy. Pairs of signals from the *S*- and *R*-esters were compared (δ_S-δ_R, Table 1), which allowed the assignment of the *S* configuration for **8**. Interestingly, unlike compounds **1–7**, **8** has a negative optical rotation, $[\alpha]_D$ −23.1°. The conjugated enynol functionality is relatively rare in the acetylenic alcohol literature. A compound with this functionality ($[\alpha]_D$ −14°) has recently been discovered from a *Petrosia* sp. by Ochi *et al.* (21). In that report, the absolute stereochemistry at the carbinol center was determined from the cd spectrum of the corresponding *p*-bromobenzoate derivative, which displayed a positive Cotton effect at 243.4 nm. Based on the exciton chirality theory (22), this should have led to the prediction of *S*, not *R* configuration as concluded by Ochi *et al.* The absolute configuration of **8** obtained from the modified Mosher's ester method is, therefore, consistent with the Cotton effect reported.

These acetylenic alcohols were evaluated in the NCI's 60-cell-line human tumor screen (23). Acetylenic alcohols **1–7** produced similar dose-response curves against the NCI panel. Although most of the cell lines showed essentially equivalent sensitivity to these compounds, two of the lines, a non-small cell lung line (H-522) and an ovarian cell line (IGROV-1), consistently showed an approximately tenfold to one hundred-fold greater sensitivity than the panel average. The subpanel and individual cell lines identifiers and the corresponding negative $\log_{10}$, GI_{50}, TGI, and LC_{50} values for **2** are provided below (see Experimental). Due to limited sample availability, alcohol **8** was tested against only these two most sensitive lines and two additional growth kinetically matched cell lines of similar histologic type. Results of testing in this subpanel indicated in vitro activity similar to **1–7**.

We have found it difficult to obtain reproducible growth of the H-522 cell line in the mouse xenograft model. Therefore, the ovarian IGROV-1 cell line was used in a preliminary in vivo study of racemic 3-hydroxyeicos-(4*E*)-en-1-yne [**1**], synthesized by a published procedure (24). In preliminary in vitro testing, the synthetic material showed activity similar to natural **1** (Table 2). Intraperitoneal treatment of athymic mice bearing intra-abdominal IGROV-1 ovarian carcinoma with a sesame oil solution of **1** (once daily doses of 50 mg/kg beginning on the day following tumor cell implantation) resulted in a small, but statistically significant ($p<0.05$; Wilcoxon test) increase in lifespan (e.g., median lifespan of treated group 36.5 days compared to 28 days for untreated controls. Thus, the synthetic racemic **1** may suffice as a representative of this series for further in vivo investigation if warranted.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were taken on a Perkin-Elmer 241 polarimeter in MeOH. Ft-ir spectra were obtained on a Perkin-Elmer 267 spectrometer. Eims spectra were measured on a VG 70-250 mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Varian VXR-500 spectrometer using $CDCl_3$ as solvent and internal standard (^{1}H, δ 7.24, ^{13}C, δ 77.0). The number of attached protons for each carbon was determined from DEPT experiments. Hplc was performed on a Waters 600E system with Rainin Microsorb (1.0×25 cm or 2.1×25 cm) columns using a Waters 900 photodiode array detector.

ANIMAL MATERIAL.—Samples of *Cribrochalina vasculum* were collected at a depth of 38 to 148 meters in the Caribbean near Egg Island, southwest corner of the Bahamas (76° 52.78 W, 25° 26.32 N), by Harbor Branch Oceanographic Institute under contract to the National Cancer Institute. Sponge samples (732 g) were kept frozen prior to extraction. The frozen sponge was ground with dry ice, extracted with H_2O at 4°; the aqueous extract was removed by centrifugation and lyophilized. The sponge residue was also lyophilized.

The dry marc (136.9 g) was then extracted overnight at room temperature with MeOH-CH_2Cl_2 (1:1), followed by MeOH. Solvents from the combined extracts were removed *in vacuo* to give a dark green residue (6.27 g). A portion of this extract (1.995 g) was partitioned between hexane and 90% MeOH. The hexane-soluble fraction (1.377 g) was further fractionated by vlc on a cyano-bonded phase (40 μm) with increasingly polar mixtures of MeOtBu/hexanes. The cytotoxic fractions were further purified by reversed-phase hplc on C_{18} columns (Rainin Microsorb, 5 μm) using MeOH-H_2O (9:1) as eluent, affording compounds **1** (3.3 mg), **2** (35.8 mg), **3** (41.9 mg), **4** (27.7 mg), **5** (12.2 mg), **6** (11.1 mg), **7** (3.0 mg), and **8** (4.1 mg). Compounds **1–6** provided ms and ^{1}H- and ^{13}C-nmr spectra consistent with literature reports (11,12).

(3S)-Hydroxyeicos-(4E)-en-1-yne [**1**].—White solid; [α]D +18.3° (*c*=0.37, MeOH).

(3S)-Hydroxydocosa-(4E,15Z)-dien-1-yne [**2**].—Colorless oil; [α]D +21.5° (*c*=1.1, MeOH).

(3S)-Hydroxy-16-methyleicos-(4E)-en-1-yne [**3**].—Colorless oil; [α]D +17.3° (*c*=0.46, MeOH).

(3S)-Hydroxy-19-methyleicos-(4E)-en-1-yne [**4**].—White solid; [α]D +22.2° (*c*=0.58, MeOH).

(3S)-Hydroxy-21-methyldocosa-(4E,15Z)-dien-1-yne [**5**].—Colorless oil; [α]D +17.9° (*c*=1.1, MeOH).

(3S)-Hydroxy-14-methyldocosa-(4E)-en-1-yne [**6**].—Colorless oil; [α]D +12.4° (*c*=0.37, MeOH).

(3S)-Hydroxyheneeicos-(4E)-en-1-yne [**7**].—White solid; [α]D +11.0° (*c*=0.21, MeOH); ^{1}H nmr ($CDCl_3$) δ 0.86 (3H, d, *J*=7 Hz, Me-21), 1.23–1.36 (28H, m), 1.77 (1H, br, OH-3), 2.04 (2H, q, *J*=7 Hz), 2.54 (1H, d, *J*=1 Hz), 4.82 (1H, br d, *J*=5.5 Hz), 5.58 (1H, ddt, *J*=15, 5.5, and 1 Hz), 5.90 (1H, dtd, *J*=15, 7, and 1 Hz); ^{13}C nmr ($CDCl_3$) δ 14.12 (Me, C-21), 22.68 (CH_2, C-20), 28.82 (CH_2), 29.18 (CH_2), 29.47 (CH_2), 29.58 (CH_2), 29.69 (CH_2), 31.92 (CH_2), 31.93 (CH_2), 62.83 (CH, C-3), 73.96 (CH, C-1), 83.32 (C, C-2), 128.29 (CH, C-4), 134.70 (CH, C-5); hreims *m/z* 306.2929 (calcd for $C_{21}H_{38}O$, 306.2923); lreims *m/z* 306 (4), 123 (22), 110 (25), 109 (40), 96 (31) 95 (40), 91 (21), 83 (27), 43 (100).

(5S)-Hydroxy-16-methyleicos-(3Z)-en-1-yne [**8**].—Colorless oil; [α]D −23.1° (*c*=0.33, MeOH); ^{1}H nmr ($CDCl_3$) δ 0.81 (3H, d, *J*=7 Hz, Me-16), 0.86 (3H, t, *J*=7.5 Hz, Me-20), 1.05 (2H, m), 1.23–1.63 (26H, m), 3.11 (1H, d, *J*=2 Hz, H-1), 4.66 (1H, dtd, *J*=7.5, 7, and 1 Hz, H-5), 5.51 (1H, ddd, *J*=11.5, 2, and 1 Hz, H-3), 5.97 (1H, ddd, *J*=7.5, 11.5, and 1 Hz, H-4); ^{13}C nmr δ 14.12 (Me, C-20), 19.71 (Me, C-21), 22.69 (CH_2), 25.07 (CH_2), 27.04 (CH_2), 27.07 (CH_2), 27.09 (CH_2), 29.52 (CH_2), 29.57 (CH_2), 29.63 (CH_2), 29.69 (CH_2), 29.99 (CH_2), 31.96 (CH_2), 32.74 (CH_2), 36.54 (CH_2, C-6), 37.09 (CH_2), 70.06 (CH, C-5), 79.48 (CH, C-1), 82.73 (C, C-2), 108.85 (CH, C-3), 147.49 (CH, C-4); hreims *m/z* 306.2919 (calcd for $C_{21}H_{38}O$, 306.2923); lreims *m/z* 306 (21), 291 (0.2), 248 (0.2), 221 (0.2), 95 (100), 81 (3), 43 (16).

MTPA ESTERS OF COMPOUNDS **2**, **3**, **6**, AND **8**.—To solutions of each of the acetylenic alcohols (1 to 3 mg) in dry pyridine (0.5 ml), were added 4 × molar excess of α-methoxy-α-trifluoromethylphenylacetic acid chloride (MTPA-Cl) and a catalytic amount (few granules) of 4-dimethylaminopyridine. The mixture was stirred at room temperature overnight under N_2. The progress of the reaction was monitored by tlc (cyano-bonded phase, hexane-*i*-PrOH, 95:5). The reaction was then quenched by removal of solvent *in vacuo*, and the residue obtained was redissolved in 0.5 ml of CH_2Cl_2 and applied to a small cyano-bonded phase column (2×4 cm) equilibrated with hexane. Vlc, eluting with hexane (40 ml), gave pure Mosher esters. Both *R*- and *S*- esters were prepared for each compound and characterized by 500 MHz ^{1}H-nmr spectral data.

ANTI-TUMOR TESTING (NCI IN VITRO SCREEN).—The tumor cell line subpanels are identified as follows: I (leukemia); II (lung, non small-cell); III (colon); IV (CNS); V (melanoma); VI (ovarian); VII (renal); VIII (prostate); IX (breast). The subpanel and individual cell line identifiers are given, along with the corresponding negative $\log_{10}$ GI_{50}, TGI, and LC_{50} values, respectively. For **2**: [I]: CCRF-CEM (9.20, 8.81, 7.21), HL-60 (TB) (7.99, 7.44, 6.89), K-562 (7.51, 7.16, 6.81), MOLT-4 (8.19, 7.57, 6.90), RPMI-8226 (8.17, 7.73, 6.89), SR (7.81, 7.26, 6.76); [II]: A549/ATCC (7.34, 7.08, 6.83), EKVX (7.33, 7.09, 6.84), HOP-62 (7.28, 7.05, 6.82), HOP-92 (7.33, 7.08, 6.84), NCI-H226 (7.52, 7.21, 6.89), NCI-H23 (7.87, 7.34, 6.96), NCI-H322M (7.27, 7.04, 6.82), NCI H-460 (7.37, 7.10, 6.82), NCI-H522 (9.94, 9.36, 8.93); [III]: COLO 205 (7.36, 7.10, 6.85), HCC-2998 (7.36, 7.10, 6.85), HCT-116 (7.60, 7.26, 6.93), HCT-15 (7.44, 7.16, 6.88), HT29 (7.51, 7.18, 6.86), KM12 (7.43, 7.15, 6.88), SW-620 (8.03, 7.37, 6.95); [IV]: SF-268 (7.41, 7.13, 6.86), SF-295 (7.35, 7.10, 6.84), SF-539 (7.35, 7.10, 6.85), SNB-19 (7.28, 7.05, 6.82), SNB-75 (7.91, 7.36, 6.97), U251 (7.39, 7.13, 6.86); [V]: MALME-3M (8.20, 7.69, 7.14), M14 (8.08, 7.40, 7.00), SK-MEL-2 (8.09, 7.63, 7.08), SK-MEL-28 (7.38, 7.12, 6.86), SK-MEL-5 (7.49, 7.19, 6.89), UACC-257 (7.49, 7.19, 6.90), UACC-62 (7.41, 7.14, 6.87); [VI]: IGROVI (9.25, 8.95, 8.66), OVCAR-3 (7.31, 7.07, 6.84), OVCAR-4 (7.73, 7.29, 6.95), OVCAR-5 (7.33, 7.09, 6.84), OVCAR-8 (7.43, 7.15, 6.88), SK-OV-3 (7.21, 7.00, 6.80); [VII]: 786-0 (7.37, 7.11, 6.86), A498 (7.23, 7.02, 6.81), ACHN (7.34, 7.10, 6.85), CAKI-1 (7.28, 7.04, 6.79), RXF-393 (7.48, 7.18, 6.88), SN12C (7.54, 7.23, 6.91), TK-10 (7.22, 7.01, 6.81), UO-31 (7.28, 7.05, 6.82), [VIII]: PC-3 (7.40, 7.13, 6.86), DU-145 (7.32, 7.08, 6.84); [IX]: MCF-7 (7.86, 7.32, 6.91), MCF7/ADR-RES (7.42, 7.14, 6.86), MDA-MB-231/ATCC (7.32, 7.08, 6.84),

HS578T (7.35, 7.06, 6.77), MDA-MB-435 (7.57, 7.24, 6.90), MDA-N (7.60, 7.27, 6.94), BT-549 (7.41, 7.14, 6.87), T-47D (8.20, 7.37, 6.94).

ACKNOWLEDGMENTS

We thank K.M. Snader, D.J. Newman, and Harbor Branch Oceanographic Institution for sponge collections. T. McCloud for extractions, A. Monks and D. Scudiero for the primary antitumor screens, G. Gray, J. Roman, and L. Pannell for mass spectral analysis, C. Hughes for technical assistance, and M. Bernart for helpful discussions.

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Received 29 December 1994