Isolation of the Furan Fatty Acid, (8Z,11Z,14Z,17Z)-3,6-Epoxyeicos-3,5,8,11,14,17-hexenoic Acid from the New Zealand Sponge Hymeniacidon hauraki

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ISOLATION OF THE FURAN FATTY ACID, (8Z,11Z,14Z,17Z)-3,6-EPOXYEICOS-3,5,8,11,14,17-HEXENOIC ACID FROM THE NEW ZEALAND SPONGE *HYMENIACIDON HAURAKI*

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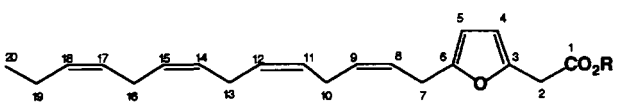
ABSTRACT.—Observed cytotoxicity of the New Zealand sponge, *Hymeniacidon hauraki*, was attributed to a furan fatty acid constituent, (8Z,11Z,14Z,17Z)-3,6-epoxyeicos-3,5,8,11,14,17-hexenoic acid [**1**].

Furan fatty acids have been previously found in a number of marine organisms including crayfish (1,2), diatoms (3), soft corals (4), ascidians (5), and scallops (5), but have been isolated most commonly from fish lipids (5–10). Reports of their isolation from plant sources are less common (11–14). We now report the isolation of the furan fatty acid, (8Z,11Z,14Z,17Z)-3,6-epoxyeicos-3,5,8,11,14,17-hexenoic acid [**1**] from the New Zealand marine sponge, *Hymeniacidon hauraki* (family Hymeniacidonidae, order Halichondrida). The acid has previously been isolated from the sponge, *Dictyonella incisa*, as the methyl ester, by methanolysis of the steryl ester form in which it occurred in the sponge (15). Both the methyl ester and the steryl ester forms showed inflammatory activity. The furan fatty acid [**1**] is responsible for the cytotoxicity of extracts of *H. hauraki* (P-388; IC_{50} 13.4 μg/ml), a property not hitherto reported.

Partitioning of the MeOH/toluene extract from the sponge between $CHCl_3$ and H_2O, followed by bioassay-guided repeated cc of the organic soluble fraction on Si gel yielded a furan acid [**1**]. The structure of **1** followed from a series of ^{13}C-, COSY, HETCOR, and DEPT nmr experiments. Nmr spectroscopic data obtained were generally in agreement with those previously published (15), but those data are for the methyl ester of **1** and a different solvent was used to obtain the ^1H-nmr spectrum.

The furan acid [**1**] decomposed before full characterization could be achieved. A second extraction of the sponge was performed under conditions designed to exclude as much light and oxygen as possible. The sponge was blended as previously, but the blender was flushed with N_2 and an antioxidant, β-carotene, was added to the extract prior to blending, in an attempt to halt decomposition of the furan acid. After reversed-phase flash cc of the extract, fractions shown to contain **1** (tlc and ^1H-nmr analysis) were recombined and rechromatographed using DIOL as the stationary phase. This led to the reisolation of **1** and completion of the characterization as the derived methyl ester **2**.

The furan acid **1** was cytotoxic against



1 R=H
2 R=CH_3

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the P-388 cell line (IC_{50} 13.4 μg/ml) and this was confirmed by comparable activity against the BSC cell line (2+ at 5 μg/disk) (see Experimental). In both assays the methyl ester [**2**] was inactive, implying that the carboxylic acid functionality is necessary for the cytotoxicity of this compound.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Eims were obtained on a Kratos MS80RFA mass spectrometer; ir spectra on a Pye Unicam SP-300 infrared spectrophotometer; and uv spectra as MeOH solutions using a Varian DMS 100 spectrophotometer. ^{13}C-Nmr spectra at 75 MHz and ^{1}H-nmr spectra at 300 MHz were recorded on a Varian XL-300 spectrometer using $CDCl_3$ solutions. Cc was performed using Si gel (Davisil 35–70 μm) and C_{17} reversed-phase material prepared by coating Si gel with *n*-octadecyltrichlorosilane after the method of Evans *et al.* (16). Mplc was performed on a Lobar DIOL column (Merck LiChroprep) using a Milton-Royal pump, Rheodyne type 50 injector, and a LKB 2212 Helirac fraction collector, with detection by an LKB 2142 differential refractometer and an LKB 2238 Uvicord II uv detector. All solvents used were either spectral grade or distilled prior to use.

ANIMAL MATERIAL.—*Hymeniacidon hauraki* (510 g wet wt) was collected in February 1987 by scuba from Leigh Reef, Leigh, New Zealand. A voucher specimen, 87LR01-05 is held at the Department of Chemistry, University of Canterbury.

EXTRACTION AND ISOLATION.—The orange sponge was stored frozen and then blended with MeOH-toluene (4:1; 2×1 liter), filtered under vacuum, and the solvent removed. The resulting cytotoxic extract (16.6 g) was partitioned between $CHCl_3$ and H_2O. The organic extract (410 mg) was chromatographed on Si gel (15 g), using a petroleum ether to EtOAc gradient to yield 10 fractions. Bioassays were used to locate the cytotoxicity. One bioactive fraction (35 mg) from this column was pure and was used for ^{1}H-, ^{13}C-, HETCOR, COSY, and DEPT nmr experiments. Other fractions, shown to contain **1**, were recombined (71 mg) for further chromatographic purification on Si gel (2 g), using a petroleum ether to EtOAc gradient as before to yield (8*Z*,11*Z*,14*Z*,17*Z*)-3,6-epoxyeicos-3,5,8,11,14,17-hexenoic acid [**1**] (37 mg) as a yellow oil: ir (smear) ν max 3420, 2960, 1720, 1575, 1410, 1230, 1020, 990, 790, 720 cm^{-1}; uv λ max (MeOH) 220, 281 nm (log ε 3.13, 2.40, respectively); ^{1}H nmr ($CDCl_3$) δ 3.66 (2H, s, H_2-2), 6.13 (1H, d, *J*=3.2 Hz, H-4), 5.93 (1H, d, *J*=3.2 Hz, H-5), 3.39 (2H, d, *J*=6.3 Hz, H_2-7), 5.54 (2H, m, *J*=6.3 and 10.6 Hz, H-8, H-9), 2.82 (6H, m, H_2-10, H_2-13, H_2-16), 5.37 (6H, m, H-11, H-12, H-14, H-15, H-17, H-18), 2.06 (2H, m, *J*=7.6 and 7.0 Hz, H_2-19), 0.96 (3H, t, *J*=7.6 Hz, H_3-20); ^{13}C nmr ($CDCl_3$) δ 174.84 s (C-1), 33.80 t (C-2), 155.66 s (C-3), 108.99 d (C-4), 105.99 d (C-5), 145.45 s (C-6), 26.28 t (C-7), 124.64 d (C-8), 130.07 d (C-9), 25.59 t (C-10), 127.78* d (C-11), 127.70 d (C-12), 25.59 t (C-13), 128.47* d (C-14), 128.57* (C-15), 25.50 t (C-16), 126.97 d (C-17), 132.01 d (C-18), 20.52 t (C-19), 14.14 q (C-20) *Sequential values may be interchanged; HETCOR, H-2 <–> $C_{33.80}$; H-4 <–> $C_{108.99}$; H-5 <–> $C_{105.99}$; H-7 <–> $C_{26.28}$; H-8, H-9 <–> $C_{124.64}$, $C_{130.07}$; H-10, H-13 <–> $C_{25.59}$; H-16 <–> $C_{25.50}$.

Reextraction of sponge.—H. hauraki (50 g) was extracted in the manner described, except that CH_2Cl_2 was used instead of toluene, the blender was flushed with N_2, and β-carotene (200 mg) was added to the sample prior to blending. The resultant extract (2.44 g) was dry-coated onto Celite (1 g) and partitioned by flash reversed-phase cc on C_{18} using a H_2O to MeOH to CH_2Cl_2 gradient. Fractions containing **1** were combined (47 mg) and rechromatographed on a DIOL Lobar column eluting with petroleum ether-EtOAc (4:1) as solvent at 5 ml/min with both ri and uv detection. A low yield of **1** (0.5 mg), pure by ^{1}H nmr, was obtained as a yellow oil.

PREPARATION OF FURAN ACID METHYL ESTER [**2**].—A sub-sample of **1** (250 μg) was dissolved in MeOH, excess CH_2N_2 in Et_2O added, and the solution left for 3 h. Tlc analysis on Si gel (petroleum ether-EtOAc [1:1]; visualization with I_2 vapor) revealed that the reaction product was a single compound (R_f=0.9). Ms *m/z* found M^+ 328.2033 ($C_{21}H_{28}O_3$ requires M^+ 328.2038).

BIOASSAYS.—*P-388 Assay.*—A 2× dilution series of the sample of interest was incubated for 72 h with P-388 [ATCC CCL 46 (Flow Laboratories)] cells. The concentration of sample required to reduce the P-388 cell growth by 50% (comparative to control cells) was determined using the absorbance values obtained when the yellow dye MTT tetrazolium was reduced by healthy cells to the purple MTT formazan. The result was expressed as an IC_{50} in μg/ml.

Antiviral assay.—Samples were pipetted onto filter paper disks (6 mm) and the solvent evaporated. The disks were placed directly onto BSC-1 cells (African Green Monkey kidney cells) grown to >90% confluence, before being transferred to a maintenance media comprising MEM containing 1.5% fetal calf serum and 1.5% methylcellulose. The BSC cells had previously been infected with either *Herpes simplex* Type 1 virus (ATCC VR 733) or *Polio* virus type 1 (Pfiser

vaccine strain). After 24 h the wells were examined (with an inverted microscope) for the size of antiviral (i.e., viral inhibition) and/or cytotoxic zones. The following scale was used for the antiviral and cytotoxicity (zone) results: ND, no discernible antiviral or cytotoxic effects; +−, minor effects located under the disk; +, antiviral or cytotoxic zone 1–2 mm excess radius from the disk (25% zone); 2+, antiviral or cytotoxic zone 2–4 mm excess radius from the disk (50% zone); 3+, antiviral or cytotoxic zone 4–6 mm excess radius from the disk (75% zone); 4+, antiviral or cytotoxic zone over the whole well (100% zone).

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