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LIPIDS FROM THE BROWN ALGA *CYSTOSEIRA BARBATA*

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Key Word Index—*Cystoseira barbata*; Phaeophyceae; brown alga; ethyl-(*Z*),(*Z*)-hexadec-10,13-dienate; (*Z*),(*Z*)-hexadec-10,13-dienal.

Abstract—Two new diunsaturated lipids, related to palmitic acid, were isolated from the brown alga *Cystoseira barbata*, one of which is toxic to mice during P388 lymphocytic leukemia tests.

As part of our program on the chemical constituents of Cystoseiraceae (Phaeophyceae) [1–4], we have examined an organic solvent extract of *Cystoseira barbata*. Our initial interest in the lipid soluble material, was prompted by significant *in vitro* activity shown by an extract of the freeze-dried alga during L 1210 tests (DE_{50} 15 μg/ml). We wish to describe here the structure of the two major products.

The CH_2Cl_2–MeOH (1:1) extract of the freeze-dried alga was fractionated by silica gel open-column chromatography, using hexane–Et_2O solvent mixtures. Compounds **1** and **2** were obtained directly and purified by HPLC on a μ-Porasil column (respectively 3% and 5% EtOAc in isooctane).

Compound **1** (10% of extract) was isolated as a yellow oil and analysed as $C_{18}H_{32}O_2$ (peak matching *m/z* obs: 280.2402, calc. 280.2400). The presence of an ester functionality was indicated by IR absorption at 1735 cm^{-1} and an Et ester was indicated from the observation of a base peak at *m/z* 88 (McLafferty rearrangement) in the mass spectrum [5]. Double bonds, with *Z* configuration, in a linear alkyl chain were supported by ^1H NMR resonances at 5.35 ppm (4H, *t*, J = 7 Hz) and an Me group included in a CH_3–CH_2–C=C arrangement was discerned by a sharp triplet signal [6]. At least, decoupling experiments gave partial support to structure **1**.

Oxidative cleavage of **1** with $NaIO_4$–$KMnO_4$ in *t*-BuOH [7] established the positions of double bonds and alkyl chain by giving a product (35%) which was identified as sebacic monoEt ester by comparison with a commercial sample. Due to purification procedures, this fatty acid was not described in an earlier publication [8] on the composition of lipids in *C. barbata*.

Compound **2** was isolated (13% of extract) as a white foam and analysed as $C_{16}H_{28}O$ (peak matching *m/z* obs: 236.2136, calc. 236.2133). It was closely related to **1** as determined by ^1H NMR. IR bands at 2680 cm^{-1} and 1710 cm^{-1} and a signal at δ9.40 (1H, *br s*) in the ^1H NMR spectrum were the main differences. Thus, **2** was concluded to be the aldehydic form of **1**.

To confirm the close relationship between the two lipids, **2** was oxidized with Ag_2O, followed by CH_2N_2 treatment to obtain the Me ester **3** which was almost identical to **1** by spectral analysis. However, final confirmation was obtained by oxidative cleavage since sebacic monoMe ester was the end product of the reaction.

It is well known that unsaturated fatty acids are active against *in vitro* P388 but not during *in vivo* tests [9] unless they are added in the diet [10]. To confirm our initial *in vitro* results, compounds **1** and **2** were submitted to *in vivo* P388 leukemia tests*. No activity was observed for **1** during these tests but **2** seems to be toxic† at medium concentrations (40 mg/kg). At least, toxicity of **2** could be related to the parodoxical involvement of fatty acids during the outcome of some tumors [11] through the possible biogenetic reduction of fatty acids to aldehydes.

EXPERIMENTAL

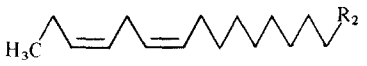
Freshly collected *C. barbata* collected at Salses, February 1983 was freeze-dried (300 g), ground to a fine powder and extracted with CH_2Cl_2–MeOH (1:1). After filtration, the MeOH was evapd and 3 g of extract (1% dry wt) were obtained. Extract (1 g) was applied to a silica gel column and elution carried out with a solvent gradient from hexane to Et_2O. Compound **1** (0.1% dry wt) was eluted with hexane–Et_2O (4:1) and compound **2** (0.13% dry wt) with hexane–Et_2O (7:3). The two metabolites were subsequently purified by HPLC, with 3% and 5% EtOAc in isooctane, respectively.

Ethyl-(Z),(Z)-hexadec-10,13-dienate (**1**). IR ν_{max}^{film} cm^{-1}: 1735; ^1H NMR (90 MHz, $CDCl_3$): δ5.35 (4H, *t*, J = 7 Hz), 4.27 (2H, *q*, J = 7.5 Hz), 2.79 (2H, *br t*), 2.35 (2H, *br t*), 2.08 (4H, *vbr t*), 1.28 (12H, *br s*), 1.20 (3H, *t*, J = 7.5 Hz), 0.95 (3H, *t*, J = 7.5 Hz); EIMS 70 eV *m/z* (rel. int.): 280 $[M]^+$ (8); 88 $C_4H_8O_2$ [McLafferty rearrangement] (100); HRMS: $C_{18}H_{32}O_2$ (*m/z* obs. 280.2402, calc. 280.2400).

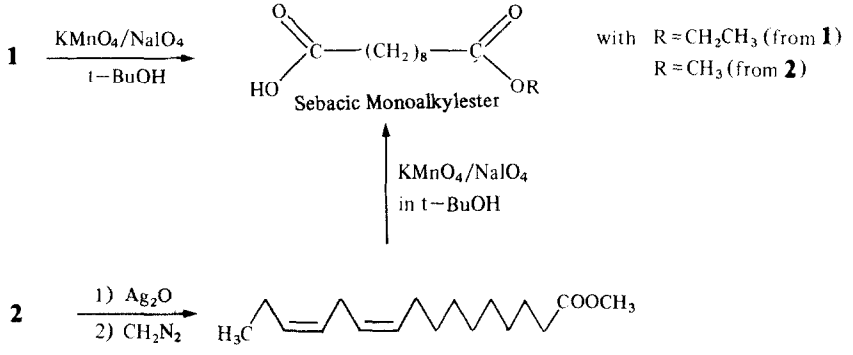
(Z),(Z)-Hexadec-10,13-dienal (**2**). IR ν_{max}^{film} cm^{-1}: 2680, 1710; ^1H NMR ($CDCl_3$): δ9.40 (1H, *br s*), 5.35 (4H, *t*, J = 7 Hz), 2.79 (2H, *br t*), 2.35 (2H, *br t*), 2.06 (4H, *vbr t*), 1.27 (12H, *br s*), 0.95 (3H, *t*, J = 7.5 Hz); HRMS: $C_{16}H_{18}O$ (*m/z* obs. 236.2136, calc. 236.2133); EIMS 70 eV *m/z* (rel. int.): 236 $[M]^+$ (12), 192 [M

*These data are the results of screening performed under the auspices of the Developmental Therapeutics program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

†Excessive deaths (> 34%) occur during these experiments and are indications of toxicity in the survival model.



1 $R_2 = COOC_2H_5$
2 $R_2 = CHO$



$-C_2H_4O]^+$ (7), 69 $[C_5H_9]^+$ (100).

Oxidative cleavage of **1**. To 5 ml of a H_2O soln of $NaIO_4$ (3.5 mmol), $KMnO_4$ (0.25 mmol) and K_2CO_3 (6 mmol) was added 70 mg of **1** in 5 ml of H_2O–*t*-BuOH (2:1). The mixture was stirred for 12 hr at room temp., at which time 100 ml of Et_2O was added. The Et_2O was collected, washed with H_2O (3 × 25 ml), dried ($MgSO_4$), filtered and evapd to yield 20 mg of starting material. The same process was conducted on the aq. phase after acid treatment to yield 25 mg of sebacic monoEt ester; IR ν_{max}^{film} cm^{-1}: 1735, 1710; ^1H NMR ($CDCl_3$): δ 10.5 (1H, *br s*), 4.27 (2H, *q*, J = 7.5 Hz), 2.36 (4H, *br t*), 1.29 (12H, *br s*), 1.20 (3H, *t*, J = 7.5 Hz).

Oxidation of **2**. Ag_2O (100 mg) and Na_2SO_4 (120 mg) were stirred with a soln of **2** (100 mg) in Et_2O (10 ml) at room temp. After 3 hr (IR ν_{max}^{film} cm^{-1}: 1710) the ppt was filtered off and the soln treated with CH_2N_2 in order to obtain the corresponding Me ester **3** (90 mg); IR ν_{max}^{film} cm^{-1}: 1735; ^1H NMR ($CDCl_3$): δ 3.50 (3H, *s*).

Oxidative cleavage of **3**. The reaction was performed as described for **1** and 30 mg of the oxidized product (sebacic monoMe ester) was obtained. IR ν_{max}^{film} cm^{-1}: 1735, 1710; ^1H NMR ($CDCl_3$): δ 10.3 (1H, *br s*), 3.50 (3H, *s*), 2.38 (4H, *br t*), 1.30 (12H, *br s*), 1.21 (3H, *t*, J = 7.5 Hz).

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