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SHORT REPORTS

7-METHOXY-9-METHYLHEXADECA-4(*E*),8(*E*)-DIENOIC ACID FROM *LYNGBYA MAJUSCULA*

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Key Word Index—*Lyngbya majuscula*; Oscillatoriaceae; fatty acid; 7-methoxy-9-methylhexadeca-4(*E*),8(*E*)-dienoic acid.

Abstract—A new fatty acid, 7-methoxy-9-methylhexadeca-4(*E*),8(*E*)-dienoic acid, has been isolated from a toxic, deep-water variety of the marine blue-green alga *Lyngbya majuscula*.

INTRODUCTION

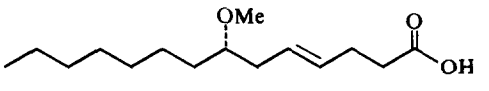
7(*S*)-Methoxytetradec-4(*E*)-enoic acid (**1**) and amides of **1** are lipophilic constituents of shallow-water varieties of the marine cyanophyte *Lyngbya majuscula* [1]. These compounds, however, were not found in a deep-water variety of the blue-green alga collected from Reefer 8 pinnacle in Enewetak lagoon. Instead two amides of 7-methoxy-9-methylhexadec-4(*E*)-enoic acid (stereochemistry and absolute configuration unknown) were isolated [2], but the free fatty acid could not be detected. From a deep-water specimen of *L. majuscula* growing on Sand Island pinnacle, Enewetak, we have now isolated a small amount of a related fatty acid (**3**). This report describes the structure determination of this new acid.

RESULTS AND DISCUSSION

Extraction of the freeze-dried alga followed by solvent partitioning, two gel filtrations and liquid chromatography of the extract gave the Me ester of the fatty acid. During the second gel filtration, for which a mixture of $CHCl_3$ and MeOH was used as eluant, the acid was accidentally converted to the Me ester **4** as shown by the appearance of a sharp singlet at δ 3.62 in the ^{1}H NMR spectrum. This signal was absent in the spectrum of the material before the second gel filtration.

The MS of the ester showed a small M^+ at *m/e* 310 and a fragment ion at *m/e* 279 for loss of OMe from the M^+. High resolution MS established that the molecular composition was $C_{19}H_{34}O_3$.

Analysis of the ^{1}H NMR spectrum in acetone-d_6 allowed us to conclude that the ester was a Me 7-methoxy-9-methylhexadec-4(*E*),8-dienoate. A singlet at δ 3.16 was attributed to a second OMe group (ether type). All 3 oxygens were now accounted for. A partially resolved doublet of quartets at δ 4.97 was assigned to an olefinic proton on a trisubstituted double bond that was allylically coupled (1.5 Hz) to a Me group at δ 1.66 and vicinally coupled (9 Hz) to a methine at δ 3.88. The Me signal at δ 1.66 was a sharp doublet and exhibited only allylic coupling (no homoallylic coupling). The methine signal at δ 3.88 was a sharp doublet (9 Hz) of triplets (6 Hz), indicating that this methine, which had to have the OMe group at δ 3.16 attached to it, was vicinally coupled to a methylene at δ 2.2. The chemical shift of the methylene at δ 2.2 suggested that it was connected to a double bond; a spin–spin decoupling experiment supported this assignment as the methylene protons were further coupled to one of two olefinic protons resonating at δ 5.46. The absorption at δ 5.46 appeared to be broad, partially resolved doublets (~15 Hz) of multiplets, suggesting that these two olefinic protons belonged to a *trans*-disubsti-



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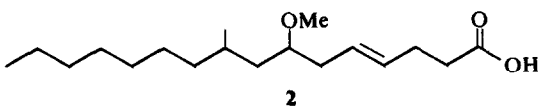

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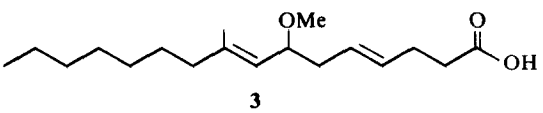

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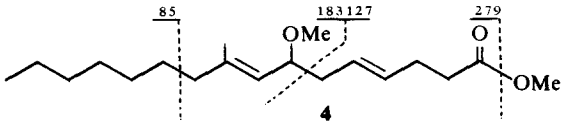

4

tuted double bond. The complex multiplet at δ 2.3 was assigned to two additional methylenes attached to the olefinic double bonds and a methylene adjacent to the ester carbonyl. A broad triplet at δ 0.88 for a Me group at the end of a *n*-alkyl chain and a broad band at δ 1.3 for a chain of 5 methylenes (integration) indicated that the ester possessed a *n*-hexyl group which had to be connected to one of the allylic methylenes resonating at δ 2.3. Attachment of the *n*-hexyl group to the allylic methylene on the trisubstituted double bond was favored on biogenetic grounds [2] since this meant that two methylenes, as in **1** and **2**, separated the 1,2-disubstituted olefinic group and the ester carbonyl.

The structure was supported by the MS which showed a base peak at *m/e* 183 and a small peak at *m/e* 127 for the fragment ions arising from cleavage of the doubly allylic C(6)-C(7) bond. Also shown was a large peak at *m/e* 85 for fission of the allylic C(10)-C(11) bond and as expected the intensities of the homologous *m/e* 71 and 99 peaks were smaller. The IR spectrum showed a strong bond at 965 cm^{-1} which confirmed the *E* geometry of the Δ^4 double bond. In the ^{13}C NMR spectrum in $CDCl_3$, the Me group on C(9) resonated at δ 16.7, denoting that the geometry of the Δ^8 double bond was *E* [3]. The structure of the fatty ester was therefore **4**.

EXPERIMENTAL

^1H NMR spectra were obtained at 100 MHz in Me_2CO-d_6 and ^{13}C NMR spectra at 25 MHz in $CDCl_3$ or Me_2CO-d_6. ^1H chemical shifts are reported in δ units (ppm) relative to Me_2CO-d_5 (δ = 2.06) as internal standard and ^{13}C chemical shifts are reported in δ units relative to $CDCl_3$ (δ = 76.9) or Me_2CO-d_6 (δ = 29.2) as internal standards. EI-MS were obtained at 70 eV.

Isolation. *L. majuscula* was collected from Sand Island pinnacle, Enewetak in February, 1976 at a depth of *ca* 10 m. The freeze-dried alga (270 g) was extracted successively with CH_2Cl_2 and EtOAc to give 5.2 g of a dark brown oil. The extract was partitioned between hexane and MeOH–H_2O (9:1). The MeOH–H_2O layer was then adjusted in concn to 65:35 and extracted with CH_2Cl_2. Evapn of the CH_2Cl_2 layer gave 1.9 g of an oil which was applied to a 190 × 2.5 cm column of Sephadex LH-20 with CH_2Cl_2–hexane (4:1). After elution with 400 ml CH_2Cl_2–hexane (4:1), a 260 ml fraction was collected and evapd to given 280 mg of a residual gum which was then subjected to gel filtration on a 114 × 2.4 cm column of Sephadex LH-20 with $CHCl_3$–MeOH (1:1). After 208 ml of solvent had passed, a 25 ml fraction was obtained to give 24 mg of the crude fatty acid. During this second gel filtration, the fatty acid was converted almost entirely to the Me ester. Final purification of the ester was achieved by HPLC on a Whatman Magnum 9 Partisil-10 column with EtOAc–CH_2Cl_2 (1:9) to yield 10 mg of Me 7-methoxy-9-methylhexadec-4(*E*),8(*E*)-dienoate (**2**) as a colorless oil: IR (neat) cm^{-1}: 1735, 956; ^1H NMR (Me_2CO-d_6): δ 5.46 (2H, *m*), 4.97 (1H, *dq*, *J* = 9 and 1.5 Hz), 3.88 (1H, *dt*, *J* = 9 and 6 Hz), 3.62 (3H, *s*), 3.16 (3H, *s*), 2.3 (6H, *m*), 2.2 (2H, *m* obscured by solvent peak), 1.66 (3H, *d*, *J* = 1.5 Hz), 1.3 (10 H, *bs s* with low field sh), 0.88 (3H, *t*, *J* = 7 Hz): ^{13}C NMR (Me_2CO-d_6): δ 13.8 (C-16), 16.2 (Me on C-9), 22.7 (C-15), 77.2 (C-7); ^{13}C NMR ($CDCl_3$): δ 14.1 (C-16), 16.7 (Me on C-9), 22.7 (C-15), 27.8, 29.2 (2 carbons), 29.6, 31.8 (C-14), 34.0, 38.0, 39.7, 51.5 (ester OMe), 55.5 (OMe on C-7), 76.9 (C-7), 127.3, 128.6, 130.1, peaks for C-9 and C = O carbons not clearly observed: MS *m/e* (rel. int.) 310 (< 1, M^+), 279 (3), 183 (100), 127 (3), 99 (3), 85 (43), 71 (21), 59 (5), 57 (2); high resolution MS *m/e* 279.2334 (calc. for $C_{18}H_{31}O_2$, 279.2324), 183.1752 (calc. for $C_{12}H_{23}O$, 183.1749), 85.1049 (calc. for C_6H_{13}, 85.1017).

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