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ANTIBACTERIAL CONSTITUENTS OF THE DIATOM *NAVICULA DELOGNEI*

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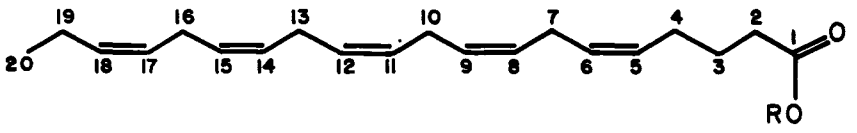
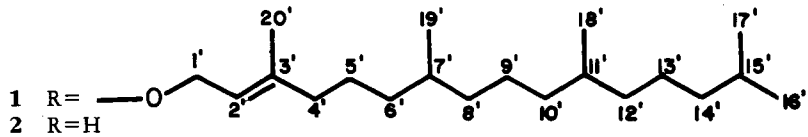
ABSTRACT.—The novel ester (*E*)-phytol (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoate (**1**); (6*Z*,9*Z*,12*Z*,15*Z*)-hexadecatetraenoic acid; (6*Z*,9*Z*,12*Z*,15*Z*)-octadecatetraenoic acid; and (6*Z*,9*Z*,12*Z*)-hexadecatrienoic acid isolated from the diatom *Navicula delognei* f. *elliptica*, show significant antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhimurium*, and *Proteus vulgaris*. β-Carotene, α-cryptoxanthin, fucoxanthin, lutein, *trans*-phytol, and plastoquinone-9 were also isolated from this diatom.

To date, there have been no reports of chemical studies on the diatom *Navicula delognei* f. *elliptica* Lobban. Since the extract of this organism showed strong antibacterial activity, we have examined its constituents and now report our findings.

Silica gel chromatography of the $CHCl_3$-soluble portion of a MeOH extract of fresh *N. delognei* f. *elliptica* afforded several fractions showing substantial antibacterial activity in disc diffusion assays. The least polar active fractions afforded an unstable ester whose structure (**1**) was established as follows. The $CHCl_3$ ir spectrum of **1** shows absorption at 1735 cm^{-1} (saturated-COOR). The compound is readily hydrolyzed by 5% KOH and yields *trans*-phytol identical with an authentic sample and an oily acid (**2**). The latter shows absorption bands at 1710 (-COOH) and 715 cm^{-1} (*cis*-CH=CH-), but none near 915 cm^{-1} (*trans*-CH=CH-) (1). The mass spectrum displays the molecular ion at *m/z* 302 ($C_{20}H_{30}O_2$). The acid (**2**), upon treatment with ethereal CH_2N_2, provided polyunsaturated methyl ester **3** whose mass spectrum, pmr spectrum, and ir





1 R= —
2 R=H
3 R=CH_3

spectrum are consistent with its assigned structure. That compound **1** is the *trans*-phytol ester of the previously reported (2) antibiotic, (5*Z*,8*Z*,11*Z*,14*Z*,17*Z*)-eicosapentaenoic acid (**2**) was confirmed by detailed analysis of the cmr spectrum of **1** (Table 1) and its comparison with that of *trans*-phytol (3). Comparison of **3** with an authentic sample confirmed its identity.

Later bioactive eluates from the silica gel chromatography of *N. delognei* f. *elliptica* extract afforded substantial quantities of (6*Z*,9*Z*,12*Z*,15*Z*)-hexadecatetraenoic acid, previously isolated from fish and shellfish (4), and (6*Z*,9*Z*,12*Z*,15*Z*)-octadecatetraenoic acid reported earlier from various sources including algal cells. Analysis by gc/ms of the methyl ester of the latter acid showed it to be contaminated with minor amounts of methyl (6*Z*,9*Z*,12*Z*)-hexadecatrienoic acid as well as **3**. The structures assigned to these acids are fully supported by ir, nmr, and mass spectral data. Only **2** has been previously reported (2) to possess antibiotic activity. Results of our antibacterial tests are summarized in Table 2.

TABLE 1. ^{13}C-Chemical Shifts[a] for **1** and *trans*-Phytol

Carbon	Compound **1**	Carbon	Compound **1**	*trans*-phytol
1	173.60 s	1′	61.25 t	59.42 t
2	33.72 t	2′	118.05 d	123.12 d
3	25.72 t	3′	142.64 s	140.27 s
4	24.82 t	4′	39.84[f] t	39.89[f] t
5	129.00[b] d	5′	39.34[f] t	39.39[f] t
6	128.22[b] d	6′	37.40[f] t	37.45[f] t
7	25.51[c] t	7′	32.77[g] d	32.81[g] d
8	128.73[b] d	8′	37.33[f] t	37.38[f] t
9	128.22[b] d	9′	37.26[f] t	37.31[f] t
10	25.59[c] t	10′	36.62[f] t	36.87[f] t
11	128.53[b] d	11′	32.65[g] d	32.72[g] d
12	128.07[b] d	12′	24.44[f] t	25.16[f] t
13	25.59[c] t	13′	24.77[f] t	24.82[f] t
14	128.07[b] d	14′	25.02[f] t	24.50[f] t
15	126.98[b] d	15′	26.55[f] d	27.99[f] d
16	25.59[c] t	16′	20.53[h] q	19.77[h] q
17	131.70[b]	17′	22.69[h] q	22.64[h] q
18	127.84[b] d	18′	22.59[h] q	22.73[h] q
19	25.02 t	19′	19.72[h] q	19.73[h] q
20	14.25 q	20′	16.34 q	16.19 q

[a]In $CDCl_3$ relative to internal TMS
[b-h]Assignments may be interchanged in columns

Other compounds isolated from this diatom include β-carotene, α-cryptoxanthin, lutein, fucoxanthin, *trans*-phytol, and plastoquinone-9. They were identified by spectral analysis, comparison with literature data, and with authentic samples where possible.

TABLE 2. Antibacterial Assay[a] Results

Organism (Source)	Compounds tested			
	1	(6*Z*,9*Z*,12*Z*,15*Z*)-Hexadecatetraenoic acid	(6*Z*,9*Z*,12*Z*,15*Z*)-Octadecatetraenoic acid	Control[b]
Enterobacter cloacea (ATCC 23355)	−	−	−	+++[f]
Escherichia coli (ATCC 25922)	−	+[c]	+	+++[f]
Klebsiella pneumoniae (ATCC 13883)	−	−	−	+++[g]
Proteus vulgaris (ATCC 13315)	+	+	++[d]	+++[g]
Salmonella typhimurium (ATCC 14028)	++	+++[e]	++	+++[f]
Serratia marescens (ATCC 8100)	−	−	−	+++[h]
Staphylococcus aureus (ATCC 25923)	+++	++	++	+++[f]
Staphylococcus epidermidis (ATCC 12228)	++	++	++	+++[f]

[a]Disc diffusion assay using ~0.5 mg test compound per standard ½ in. disc.
[b]Control antibiotic 0.1 mg per standard ½ in. disc.
[c]+ zone of inhibition noticeable
[d]++ zone of inhibition >2 mm
[e]+++ zone of inhibition >4 mm
[f]Ampicillin
[g]Tetracycline
[h]Chloramphenicol

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mass spectra were obtained with a Perkin-Elmer RMU-6D instrument. Ir spectra were recorded on a Perkin-Elmer 727B instrument. A Waters Associates hplc unit equipped with 440 absorbance and R401 refractive index detectors and employing a μ-Porasil 30 cm×3.9 mm column was used. Analytical and preparative tlc was performed with precoated silica gel G plates (Kieselgel 60, F-254). Pmr spectra were recorded on a Varian XL200 instrument using TMS as internal standard.

Fresh *Navicula delognei* f. *elliptica*[1] (1.2 kg), collected in May 1982, at Lepreau Ledges, New Brunswick, was immersed in Me_2CO for 2 h and then extracted in a Soxhlet apparatus with MeOH (6 liters) for 48 h. The solvent was evaporated in vacuo at room temperature, and the dark green residue (32 g, 2.7% wet weight) was dissolved in H_2O (500 ml), filtered through Celite, and extracted with $CHCl_3$(3×500 ml). The $CHCl_3$ extract upon evaporation yielded a crude green extract (16 g) that showed antibacterial activity and was chromatographed on a column of silica gel G (450 g) using $CHCl_3$ and $CHCl_3$-MeOH as eluant. The following compounds were eluted in order. β-Carotene (112 mg) was identified by comparison with an authentic sample and literature data (6).

Plastoquinone-9: (16 mg) was purified by preparative tlc (Rf=0.5, 6% EtOAc in hexane) and hplc (3% EtOAc in hexane) and showed spectral properties consistent with literature data (7).

Compound **1**: (118 mg) obtained as a colorless oil, was purified by preparative tlc (Rf=0.5, 6% EtOAc in hexane) and hplc (4% EtOAc in hexane): ir ($CHCl_3$) 1735, 1460, 1385, 1170 cm^{-1}; pmr ($CDCl_3$) δ 0.86 (d, 6H, J=6 Hz, 18′, 19′ CH_3), 0.88 (d, 6H, J=6 Hz, 16′, 17′ CH_3), 1.00 (t, 3H, J=8 Hz, 20 CH_3), 1.1-1.5 (m, 19H), 1.71 (d, 2H, J=8 Hz, 3 CH_2), 1.72 (s, 3H, 20′ CH_3), 2.10 (m, 6H, 4, 4′, 19 CH_2), 2.35 (t, 2H, 2 CH_2), 2.86 (bq, 8H, 7, 10, 13, 16 CH_2), 4.62 (d, 2H, J=7 Hz, 1′ CH_2), 5.40 (m, 11H, 2′, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18 CH).

Triglycerides (3.5 g) were recovered from fractions 44-52 and were not pursued.

Trans-phytol: (75 mg) was recovered from fractions 53-61 and was identified by comparison of spectra with literature data (8).

(6Z,9Z,12Z,15Z)-Hexadecatetraenoic acid (1.1 g) and (6Z,9Z,12Z,15Z)-octadecatetraenoic acid (0.6 g): were isolated after rechromatography on a silica gel G column (Me_2CO-hexane, 1:4) and purified by reverse phase preparative tlc (KC 18F, $CHCl_3$-acetonitrile, 1:9) and hplc (MeOH-$CHCl_3$, 1:14). They were characterized as their oily methyl esters following treatment with ethereal CH_2N_2.

(6Z,9Z,12Z15Z)-Octadecatetraenoic acid methyl ester: ms *m/z* 290 (M^+, $C_{19}H_{30}O_2$); ir (neat) 1740 (ester), 715 (*cis*-CH=CH-) cm^{-1}; pmr ($CDCl_3$) δ 1.00 (t, 3H, J=8 Hz, 18CH_3), 1.45 (m, 2H, 4CH_2), 1.72 (m, 2H, 3CH_2), 2.10 (m, 4H, 5, 17CH_2), 2.40 (t, 2H, J=8 Hz, 2CH_2), 2.85 (bq, 6H, 8, 11, 14CH_2), 3.70 (s, 3H, OCH_3), 5.40 (d, 8H, 6, 7, 9, 10, 12, 13, 15, 16CH).

(6Z,9Z,12Z,15Z)-Hexadecatetraenoic acid methyl ester: ms *m/z* 262 (M^+, $C_{17}H_{26}O_2$); ir (neat) 1740 cm^{-1} (ester), 710 (*cis*-CH=CH-) cm^{-1}; pmr ($CDCl_3$) δ 1.45 (m, 2H, 4CH_2), 1.70 (bs, 2H, 3CH_2), 2.10 (m, 2H, 5CH_2), 2.40 (bs, 2H, 2CH_2), 2.85 (bq, 6H, 8, 11, 14CH_2), 3.71 (s, 3H, OCH_3), 5.10 (m, 2H, 16CH_2), 5.40 (m, 7H, -CH=CH-) ppm.

α-Cryptoxanthin (11 mg) and lutein (9 mg): were recovered from fractions following the elution of a free sterol mixture (185 mg) and were separated and purified by preparative tlc (EtOAc-hexane, 3:7) and identified from spectral data (6). Fucoxanthin (2.1 g), recovered from the last fractions, was identified by comparison with literature data (9).

HYDROLYSIS OF COMPOUND **1**.—Compound **1** (25 mg) was treated with aqueous ethanolic KOH (5%, 15 ml) under N_2 for 1 h. Dilution with H_2O (50 ml) and extraction with $CHCl_3$ (3×50 ml) afforded, after drying and evaporation, a residue (12 mg) which was purified by preparative tlc to furnish pure *trans*-phytol (10 mg) as a colorless oil. The aqueous layer was acidified with HOAc and extracted with Et_2O (3×50 ml). The ethereal extract, after drying (anhydrous $MgSO_4$) and evaporation, yielded gc and tlc homogeneous (5Z,8Z,11Z,14Z,17Z)-eicosapentaenoic acid (**2**), whose methyl ester (**3**) (by CH_2N_2) had the following spectral characteristics: ir (neat) 3000, 1740, 1450, 1270, 715 cm^{-1}; pmr ($CDCl_3$) δ 0.98 (t, 3H, J=8 Hz, 20CH_3), 1.72 (m, 2H, 3CH_2), 2.12 (m, 4H, 4, 19CH_2), 2.34 (t, 2H, J=8 Hz, 2CH_2), 2.85 (bq, 8H, 7, 10, 13, 16CH_2), 3.68 (s, 3H, OCH_3), 5.39 (m, 10H, 5, 6, 8, 9, 11, 12, 14, 15, 17, 18CH); ms *m/z* 316 (M^+, $C_{21}H_{32}O_2$).

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[1]Tubes of this species contained trace amounts of cohabitants, *N. rusticensis* Lobban and *N. smithii* (C.Ag.) van Heurck.

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