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ISOTACTIC POLYMETHOXY-1-ALKENES FROM THE BLUE-GREEN ALGA *TOLYPOTHRIX CONGLUTINATA* VAR. *CHLORATA*

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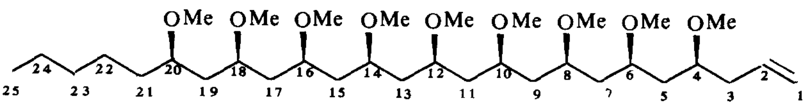
Key Word Index—*Tolypothrix conglutinata* var. *chlorata*; Scytonemataceae; blue-green alga; isotactic polymethoxy-1-alkenes; structural determination.

Abstract—4(*S**),6(*S**),8(*S**),10(*S**),12(*R**),14(*R**),16(*R**),18(*R**),20(*R**)-Nonamethoxy-1-pentacosene and smaller amounts of the isotactic homologs, 4,6,8,10,12,14,16,18,20,22-decamethoxy-1-heptacosene and 4,6,8,10,12,14,16,18-octamethoxy-1-tricosene, are novel lipophilic constituents of the toxic blue-green alga *Tolypothrix conglutinata* var. *chlorata* Ghose from Fanning Atoll.

INTRODUCTION

Toxic *Tolypothrix conglutinata* var. *chlorata* Ghose [1] from Fanning Island exhibits activity against P-388 lymphocytic leukemia in mice [2]. During isolation of the anticancer compound, tolytoxin A, a non-toxic mixture of 3 novel lipids was obtained. We report here that the major lipid in this mixture is an unusual isotactic acetogenin, 4(*S**),6(*S**),8(*S**),10(*S**),12(*R**),14(*R**),16(*R**), 18(*R**),20(*R**)-nonamethoxy-1-pentacosene (**1**).



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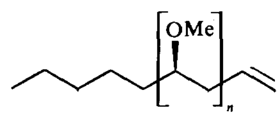


2 n = 10
3 n = 8

The two minor components are probably the isotactic homologs 4,6,8,10,12,14,16,18,20,22-decamethoxy-1-heptacosene (**2**) and 4,6,8,10,12,14,16,18-octamethoxy-1-tricosene (**3**).

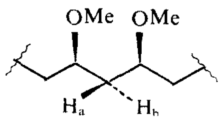
RESULTS AND DISCUSSION

The chemical ionization (CI) MS of the lipid mixture showed strong $M^+ + 1$ peaks at *m/e* 621, 679 and 563 and the relative intensities of the 3 ions indicated that the mixture was composed of mainly **1** (80%) with smaller amounts of **2** (15%) and **3** (5%). Fragment ion peaks were present in the CI-MS spectrum for successive losses of MeOH from each $M^+ + 1$ ion. Peaks at *m/e* 589, 557, 525, 493, 461, 429, 397, 365 and 333, for example, represented the successive losses of nine MeOH molecules from the $M^+ + 1$ ion of **1** (*m/e* 621) and suggested that **1** possessed at least 9 OMe groups. Only 8 of the 10 possible fragment ion peaks, however, could be readily discerned for the similar successive losses of 10 MeOH molecules from the $M^+ + 1$ ion of **2** (*m/e* 679) and only one of the 8 peaks could be seen for the successive losses of 8 MeOH molecules from the $M^+ + 1$ ion of **3** (*m/e* 563). M^+ for **1**, **2** and **3** were not observed in the corresponding EI-MS of the mixture, but prominent fragment ions were present for losses of 2, 3 and 4 MeOH molecules from the M^+ ions. High resolution mass measurements of the ions at *m/e* 556, 524 and 492 showed that **1** had the molecular formula $C_{25}H_{50}O_9$. Similarly high resolution mass measurements of the ions at *m/e* 614, 582 and 550 showed that **2** had the elemental composition $C_{27}H_{54}O_{10}$. Accurate high resolution mass measurements, however, could not be made on the $M^+ - 2MeOH$, $M^+ - 3MeOH$ and $M^+ - 4MeOH$ ions of **3** in this mixture.

The 100 MHz ^1H NMR spectrum exhibited a doublet ($J_{trans} = 16$ Hz) of doublets ($J_{cis} = 11$ Hz) of 1:2:1 triplets ($J = 6$ Hz) at δ 5.85 for the C-2 methine proton and broad (due to geminal coupling and allylic coupling) doublets at δ 5.11 ($J_{trans} = 16$ Hz) and 5.09 ($J_{cis} = 11$ Hz) for the methylene protons on C-1 of **1**, **2** and **3**. The broad triplet at δ 2.32 ($J = 6$ Hz) was assigned to the C-3 methylene protons of compounds **1**, **2** and **3**. The methine protons on C-4, C-6, C-8, C-10, C-12, C-14, C-16 and C-18 of **1**, **2** and **3**, C-20 of **1** and **2**, and C-22 of **2** appeared to be magnetically equivalent as all of these hydrogens resonated at δ 3.42 ($J = 6$ Hz) as a 1:4:6:4:1 quintet. Doublets ($J_{gem} = -14$ Hz) of triplets ($J = 6$ Hz) at δ 1.82 and 1.60 were attributed to non-equivalent methylene protons on C-5, C-7, C-9, C-11, C-13, C-15 and C-17 of **1**, **2** and **3**, C-19 of **1** and **2** and C-21 of **2**. The C-21, C-22, C-23 and C-24 methylene protons of **1**, the C-23, C-24, C-25 and C-26 methylene protons of **2**, and the C-19, C-20, C-21 and C-22 methylene protons of **3** all resonated as a broad, complex multiplet at δ 1.3. The broad triplet at δ 0.89 ($J = 7$ Hz) was assigned to the terminal Me group of **1** (C-25), **2** (C-27), and **3** (C-23) and the large singlet at δ 3.32 was ascribed to most of the OMe protons for the 3 compounds. The small singlet at δ 3.35 was due to a slightly different OMe group, presumably the one on C-4 of **1**, **2** and **3**.

The ^1H NMR assignments were confirmed by spin-spin decoupling experiments. Irradiation of the multiplet for the C-2 proton reduced the C-1 signals to singlets and the C-3 signal to a doublet. Irradiation of the quintet at δ 3.42 also collapsed the C-3 signal to a doublet, but more dramatically reduced the doublets of triplets at δ 1.82 and 1.60 to doublets ($J_{gem} = -14$ Hz).

In compounds **1**, **2** and **3** the OMe groups must be isotactic, i.e. all on the same side of the carbon chain. If the OMe groups were syndiotactic (alternating arrangement), we would have expected to see a triplet representing all of the magnetically equivalent methylene protons on C-5, C-7, C-9, C-11, C-13, C-15 and C-17, of **1**, **2**, **3**, C-19 of **1** and **2**, and C-21 of **2**. If the OMe groups were heterotactic (random-type arrangement) then two signals, one a set of two doublets of triplets for the magnetically non-equivalent methylene protons H_A and H_B in partial structure **4** and the other a triplet for the magnetically equivalent methylene protons H_A and H_B in partial structure **5**, would have been seen. The arguments presented above are supported by the following examples. In the 100 MHz ^1H NMR spectrum of *E*-1-chlorotridec-1-ene-6(*R*),8(*R*)-diol [3], the signal for the C-7 methylene is a triplet ($J = 6.5$ Hz), actually the A_2 pattern of an A_2X_2 type spectrum, and remains a triplet even at 360 MHz or in the presence of Eu(fod)$_3$. In the ^1H NMR spectrum of 3(*S**),5(*S**),8-trichloro-2,6-dimethyl-1,6(*E*)-octadiene, the C-4 methylene protons are magnetically equivalent, resulting in a triplet ($J = 7.3$ Hz); in the 3(*S**),5(*R**) diastereoisomer, however, the C-4 methylene protons are non-equivalent and two doublets of doublets of doublets are observed ($J = -14.5$, 8, 6 Hz) [4].



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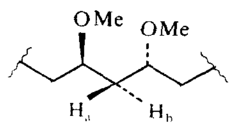

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Further support of the structures for **1**, **2** and **3** was obtained from the ^{13}C NMR spectrum of the mixture (Table 1) which showed 5 signals at δ 14.05, 22.60, 24.53, 32.02 and 33.43 that agreed well with calculated values [5] for a *n*-pentyl group. Since no other carbon signals could be seen in this region, all 3 compounds must contain a *n*-pentyl group.

EXPERIMENTAL

^1H NMR and ^{13}C NMR spectra were obtained on a 100 MHz spectrometer equipped with a Fourier transform system. ^1H chemical shifts are reported in δ units (ppm) relative to TMS ($\delta = 0$) and ^{13}C chemical shifts in δ units relative to the solvent ($CDCl_3$, δ 76.9). EI-MS were recorded at 70 eV. CI-MS were obtained using methane as the reactant gas.

Identification of alga. A microscopic examination of the cyanophyte shows that its morphology is consistent with the description of *Tolypothrix conglutinata* var. *chlorata* Ghose [1]. A voucher specimen has been retained.

Isolation. Wet *T. conglutinata* var. *chlorata* (32.4 g dry wt), collected from the wall of a shed near the Cable Station, Fanning Island in April 1977, was extracted with MeOH. The extract was evapd and the residue distributed between EtOAc and H_2O. Evapn of the EtOAc layer gave 786 mg of an oil, 607 mg of which were partitioned between *n*-hexane and MeOH–H_2O (9:1). The MeOH–H_2O layer was then adjusted in concn to 3:1 and extracted with CCl_4. The CCl_4 extract was evapd to give 123 mg of an oil, 103 mg of which were subjected to gel filtration on a 1.4 cm × 1.2 m column of Sephadex LH-20 with $CHCl_3$–MeOH (1:1). The fraction eluted from 103 to 122.5 ml contained 34 mg of a toxic gum. Further purification of 31 mg of this gum was achieved by HPLC on μ-Bonapak-C_{18} with acetonitrile and H_2O (3:1) to give 4.7 mg of a 16:3:1 mixture of **1**, **2** and **3** (eluted after the toxin) as a greenish-brown gum which was decolorized by sublimation (145°, 0.01 mm). CI-MS *m/e* (rel. int.): 679 (21, $M^+ + 1$ for **2**), 647 (17, 679 − MeOH), 621 (100, $M^+ + 1$ for **1**), 615 (1, 679 − 2MeOH), 589 (56, 621 − MeOH), 583 (1, 679 − 3MeOH), 563 (6, $M^+ + 1$ for **3**), 557 (6, 621 − 2MeOH), 551 (3, 679 − 4MeOH), 531 (7, 563 − MeOH), 525 (6, 621 − 3MeOH), 519 (7, 679 − 5MeOH), 493 (8, 621 − 4MeOH), 487 (3, 679 − 6MeOH), 461 (9, 621 − 5MeOH), 455 (6, 679 − 7MeOH), 429 (21, 621 − 6MeOH), 423 (2, 679 − 8MeOH), 397 (8, 621 − 7MeOH), 365 (4, 621 − 8MeOH), 331 (4, 621 − 9MeOH). High resolution EI-MS *m/e* 614.4775

Table 1. ^{13}C NMR data for polymethoxy-1-alkenes

Chemical shift, δ		Carbon assignment		
Obs.*	Calcd. [5]	1	2	3
14.05	13.86	25	27	23
22.60	22.65	24	26	22
24.53	26.02	22	24	20
32.02	32.65	23	25	21
33.43	34.16	21	23	19
37.7	36.60	3	3	3
38.0	38.11	5, 19	5, 21	5, 17
38.18	38.36	7, 9, 11, 13, 15, 17	7, 9, 11, 13, 15, 17, 19	7, 9, 11, 13, 15
56.15		OMe†	OMe†	OMe†
75.17	71.38	6, 8, 10, 12, 14, 16, 18	6, 8, 10, 12, 14, 16, 18, 20	6, 8, 10, 12, 14, 16
77.20	74.45	4 or 20	4 or 22	4 or 18
78.81	74.45	4 or 20	4 or 22	4 or 18
117.09		1	1	1
134.26		2	2	2

* In ppm relative to the solvent $CDCl_3$ (δ 76.9 from TMS).
† All of the methoxyl carbons have the same chemical shift.

(calcd. for $C_{35}H_{66}O_8$: 614.4740), 582.4492 (calcd. for $C_{34}H_{62}O_7$: 582.4479), 556.4335 (calcd. for $C_{32}H_{60}O_7$: 556.4338), 550.4199 (calcd. for $C_{33}H_{58}O_6$: 550.4218), 524.4094 (calcd. for $C_{31}H_{56}O_6$: 524.4075), 492.3820 (calcd. for $C_{30}H_{52}O_5$: 492.3801). ^{1}H NMR ($CDCl_3$): δ 0.89 (*br t*, 3H, J = 7 Hz), 1.3 (*br m*, 8H), 1.6 (*dt*, ~8H by integration, J = −14 and 6 Hz), 1.82 (*dt*, ~8H by integration, J = −14 and 6 Hz), 2,32 (*br t*, 2H, J = 6 Hz). 3.32 (*s*, ~24H by integration), 3.35 (*s*, 3H), 3.42 (1:4:6:4:1 quintet, ~9H by integration J = 6 Hz), 5.09 (*br d*, 1H, J = 11 Hz), 5.11 (*br d*, 1H, J = 16 Hz), 5.85 (*ddt*, 1H, J = 16, 11 and 6 Hz). ^{13}C NMR ($CDCl_3$), see Table 1.

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