Phytochemistry, 1980, Vol. 19, pp. 143–144. © Pergamon Press Ltd. Printed in England. 0031–9422/80/0101–0143 $02.00/0

STRUCTURAL ASSIGNMENT OF TWO HENEICOSAHEXAENE ISOMERS BY ^{13}C NMR SPECTROSCOPY*

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(*Received* 15 *March* 1979)

Key Word Index—*Fucus vesiculosus*; *Spongomorpha arcta*; *Alaria esculenta*; *Agarum cribrosum*; marine algae; ^{13}C NMR; hydrocarbons; heneicosahexaene isomers.

INTRODUCTION

Since the first report of all *cis*-3,6,9,12,15,18-heneicosahexaene (**1**) (Δ^3 HEH) from the diatom *Skeletonema costatum* [1] this unusual hydrocarbon and the isomer, all *cis*-1,6,9,12,15,18-heneicosahexaene (**2**) (Δ^1 HEH), have been reported from a variety of other marine and freshwater algae including a red [2] and various greens [3–5], browns [3–7], diatoms [4, 8] and phytoplankton [4, 8]. Usually identification is based upon MS, which on its own, cannot unequivocally distinguish between these isomers or provide information concerning the nature and position of the double bonds [3, 5]. The ^{13}C NMR spectra of these hydrocarbons have now been assigned and the data used to characterize the heneicosahexaene isomers from *Alaria esculenta* (L.) Grev. and *Agarum cribrosum* (Mert.) Bory.


1


2

RESULTS AND DISCUSSION

The brown alga *Fucus vesiculosus* L. is known to produce Δ^1 HEH (**2**) [6]. The hydrocarbon, obtained following repeated column and TLC, showed 16 signals in the ^{13}C NMR spectrum. The most intense resonances at δ_c 25.69 and 25.59 (Table 1), integrating for *ca* 4 carbon atoms, are characteristic signals for methylene carbons shielded by two allylic *cis*-double bonds [9, 10] and are assigned to C-8, C-11, C-14 and C-17. Based on the chemical shift parameters evaluated for the effect of *cis*-double bonds on one another [11], some olefinic resonances can be assigned (Table 1). The resonances for C-18 through C-21 were assigned by comparison with published data for the model compounds *cis*-3-hexene [10] and linolenic acid (**3**) [9]. The allylic carbon at C-20 in **2** is shielded (δ_c 20.6) compared with the corresponding carbon in a saturated hydrocarbon (δ_c 22.9), and the olefinic carbons C-18 and C-19 appear almost at the same chemical shift as the corresponding carbons in **3**. The signals for C-1 through C-4 of **2** can be assigned by direct comparison with the published data on 1-octene [10].

Table 1. ^{13}C NMR chemical shift data for Δ^1 HEH, Δ^3 HEH and model compounds

			δ_c TMS*				
1-Octene†		Δ^1 HEH **2**		Linolenic acid **3**‡		Δ^3 HEH **1**	
C-1	113.2	C-1	114.56			C-1	14.26
C-2	138.2	C-2	138.71			C-2	20.57
C-3	33.2	C-3	33.38			C-3	132.05
C-4	28.4	C-4	28.92			C-4	127.04
C-5	28.3	C-5	26.73			C-5	25.55
		C-6	129.97			C-6	128.58§
		C-7	128.53			C-7	128.27§
		C-8	25.69**			C-8	25.63
		C-9	128.23§			C-9	128.14§
		C-10	128.03§			C-10	127.89§
		C-11	25.59**			C-11	25.63
		C-12	128.03§			C-12	127.89§
		C-13	128.23§			C-13	128.14§
		C-14	25.59**			C-14	25.63
		C-15	128.03§			C-15	128.27§
		C-16	128.53§			C-16	128.58§
		C-17	25.59§	C-14	25.3	C-17	25.55
		C-18	127.09	C-15	127.0	C-18	127.04
		C-19	132.04	C-16	131.5	C-19	132.05
		C-20	20.61	C-17	20.3	C-20	20.57
		C-21	14.26	C-18	14.0	C-21	14.26

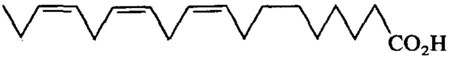
*Pulse Fourier transform spectra recorded at 30° in $CDCl_3$ at 25.16 MHz, 5 kHz width, 8 K transform, data accuracy ±0.6 Hz, internal ^{2}H pulse lock to solvent. Only the relevant positions in the model compounds are included.

†Data reported in [10].

‡Data reported in [9].

§These assignments may be interchanged.

**These assignments may be interchanged.


3

It is particularly significant that the chemical shift values for C-3 and C-4 in **2** are almost the same as those for the corresponding carbons in 1-octene, since it establishes that the next double bond in the hydrocarbon is at least two bonds removed [12] from C-3 (i.e. $\Delta^{1,6}$) and precludes other isomers (e.g. $\Delta^{1,4}$, $\Delta^{1,5}$) which are theoretically possible [5]. In accord with this substitution pattern ($\Delta^{1,6}$) in **2**, C-5 is shielded (δ_c 26.7) by the allylic *cis*-double bond at C-6, and hence

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* NRCC No. 17749.

only structure **2** will accommodate the 4 remaining methylene-interrupted double bonds.

The heneicosahexaene from the green alga *Spongomorpha arcta* (Dillw.) Kütz. has been reported to possess structure **1** on the basis of MS and IR evidence as well as GLC analysis of the ozonolysis products [3, 5]. The ^{13}C NMR spectrum of this isomer displayed only 10 signals due to the plane of symmetry through C-11. All the olefinic resonances could be distinguished and several have been assigned (Table 1) but the distinctive resonances associated with the vinyl group in **2** were absent. The resonances for the methylene carbons shielded by two allylic *cis* double bonds appeared as intense signals at δ_c 25.55 and 25.63 (ratio *ca* 2:3), and based on the intensity ratio of the signals and the symmetry of the molecule, the higher field signal is assigned to C-5 and C-17 and the lower field signal to C-8, C-11 and C-14. The first 4 carbons at each end of the hydrocarbon chain are readily assigned by comparison with the 4 terminal carbons of **3** (Table 1).

Neutral extracts of the brown algae *Alaria esculenta* and *Agarum cribrosum* yielded very similar hydrocarbon fractions containing pentadecane and a heneicosahexaene isomer. The ^{13}C NMR spectra of the polyunsaturated hydrocarbon from each source were the same and identical to that of Δ^1 HEH (**2**).

A chemotaxonomic picture is emerging [4,5,8]: brown algae appear to produce only the Δ^1 isomer, green algae and diatoms produce the Δ^3 isomer, and with one exception [2], red algae do not produce any significant amounts of polyunsaturated hydrocarbons. Although hydrocarbons are probably biosynthesized by decarboxylation of the appropriate fatty acid, no 22:6 fatty acids were found in *F. vesiculosus*, *Alaria esculenta* and *Agarum cribrosum* collected from the same area at about the same time [13].

EXPERIMENTAL

Freshly collected algae from the Bay of Fundy, N. S. were cleaned, washed and air-dried in the laboratory. Samples were identified by C. J. Bird of the Atlantic Regional Laboratory and voucher specimens deposited in the herbarium (NRCC). The dried algae were extracted with Et_2O–CH_2Cl_2 (1:1; 10 ml/g dried algae) and the extract washed with N NaOH, N HCl and H_2O. The hydrocarbons were separated by chromatography of Si gel (C_6H_{14}) and further purified by chromatography on $AgNO_3$(20%)-Si gel ($C_6H_{14} \rightarrow Et_2O$). In the purification of hydrocarbons from *F. vesiculosus*, an extra TLC step (20 cm × 20 cm × 0.2 mm; C_6H_{14} elution × 2) was required before Ag^+ chromatography. Fractions were monitored by GLC (1.5% OV-1; 2 m × 4 mm; temp. programmed 80° for 8 min → 250° at 4°/min injection port 260°, detector 265°; He carrier, 1.6 kg/cm^2; flow rate 20 ml/min; FID) and GC–MS, GLC conditions as above; glass jet separator; 70 eV ionising energy; source temp. 250°; scan limits 60–500 amu.

Following these procedures *F. vesiculosus* yielded **2** as a colourless oil. MS (probe) 70 eV *m/e* (rel int): 284 [M$^+$] (2%), 255 [M$^+$−29] (2%), 243 (M$^+$−41] (1%), 241 [M$^+$−43] (2%), 215 [M$^+$−69] (5%), 67 [M$^+$−217] (100%). $\lambda_{max}^{C_6H_{14}}$ end absorption only. ^{13}C NMR data see Table 1. Similar extraction of *S. arcta* yielded **1**, MS identical to that reported [1]. $\lambda_{max}^{C_6H_{14}}$ end absorption only. ^{13}C NMR data see Table 1.

Extraction of *Alaria esculenta* as described above gave a hydrocarbon fraction containing mainly pentadecane (*RR*$_t$, MS) and the polyunsaturated hydrocarbon **2**, obtained as a colourless oil following Ag^+ chromatography. MS: (probe) 70 eV 284.2504 [M$^+$] $C_{21}H_{32}$ requires 284.2508. $\lambda_{max}^{C_6H_{14}}$ end adsorption only. $\nu_{max}^{thin\ film}$ cm^{-1}: 910, 990, 1640 (CH_2=CH—) 720, 1650 (*cis*-HC=CH—) ^{13}C NMR data as for **2** in Table 1. The hydrocarbon fraction from *Agarum cribrosum* also contained pentadecane and **2** (*RR*$_t$ comparison authentic samples), ^{13}C NMR data as for **2** in Table 1.

Acknowledgements—I would like to thank Mrs. Cheryl Craft, Mr. Lai Szeto and Mr. David M. Saunders for excellent technical assistance and Dr. John Walter and Mr. D. G. Smith for recording the NMR spectra.

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