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Phytol-Derived C_{19} Di- and Triolefinic Hydrocarbons in Marine Zooplankton and Fishes*

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ABSTRACT: Three phytol-derived olefinic hydrocarbons have been isolated from marine zooplankton and fishes. Their structures have been determined by ultraviolet, infrared, nuclear magnetic resonance, and mass spectrometry and by combined gas chromatography and mass spectrometry of their ozonolysis products. They are the 2,10- and 5,10-diene and the 2,6,10-triene analogs of pristane (2,6,10,14-tetramethylpentadecane). The presumed mode of formation of these and related olefins and their fate in the marine food chain and in marine sediments is discussed. Because of their relative stability, these and related hydrocarbons provide tracers for the study of dynamic processes in the marine food chain. These olefins are not present in ancient sediments and in petroleum; therefore, they are valuable markers for the distinction between marine hydrocarbons derived from organisms and from oil pollution.

Chromatographic analysis of the lipids from marine zooplankton and from fish livers has yielded a saturated hydrocarbon fraction, consisting of pristane (2,6,10,14-tetramethylpentadecane, I) (Blumer *et al.*, 1963, 1964) with traces of normal alkanes and an unsaturated fraction containing squalene (Blumer, 1967) and a complex mixture of C_{19} and C_{20} olefins. We have previously reported the structures of three C_{19} monoolefins (II–IV) (Blumer and Thomas, 1965b) and four C_{20} diolefins (V–VIII) (Blumer and Thomas, 1965a). We wish to report the structures of three closely related C_{19} di- and triolefins (IX–XI). The gas chromatographic retention indices of I–XI are listed in Table I to facilitate their determination in other natural products.

The three unknown olefins have been identified in mixed zooplankton from the Gulf of Maine. They occur in *Calanus finmarchicus*, *Calanus hyperboreus*, and *Calanus glacialis;* the same compounds have been found in the liver of the herring (*Clupea harengus L.*), of the basking shark (*Cetorhinus maximus Gunnerus*) and, commonly, in other members of the marine food chain. Because of their low concentration in marine lipids, between 1 and 50 ppm, isolation in sufficient quantities for structural elucidation is difficult. A better source is crude pristane this is obtained commercially by molecular distillation of basking shark lipids. From that source we have isolated the three corresponding olefins. Their gas chromatographic retention indices agree with those of the olefins in native basking shark liver oil and in zooplankton to better than ± 2 units (see Table I). If isomerization of the olefins had occurred during preparation of the commercial pristane, a large shift in retention indices, especially on the more polar columns would be evident. The identity of the olefins from the three sources is conclusively proven by their identical retention parameters in liquid and gas chromatography.

The olefins are concentrated by frontal analysis of the crude pristane over deactivated silica gel; the concentrates are further separated by repeated adsorption chromatography on the same adsorbent. Again, gas chromatography on several substrates was used to ensure the identity of the products with the starting material; this insures against the possibility that the olefins might be artifacts produced on the adsorbent, *e.g.*, by isomerization. Complete chromatographic resolution, especially of the two isomeric dienes, is difficult and is best achieved by trapping of effluent samples from an analytical gas chromatography column. A strongly polar column (*e.g.*, FFAP) of at least 3000 theoretical plates is necessary for the resolution.

Molecular weights of the three unknowns were obtained by mass spectrometry; IX and X are isomeric C_{19} diolefins (mol wt 264), XI is a C_{19} triolefin (mol wt 262). This agrees with their gas chromatographic behavior and the greater ΔI for XI on the most polar columns (Kovats, 1958; Wehrli and Kovats, 1959). Because of hydrogen rearrangement during excitation, the mass spectra are inadequate to locate the position of the double bonds. This situation is commonly encountered for alkenes not containing tetrasubstituted double bonds.

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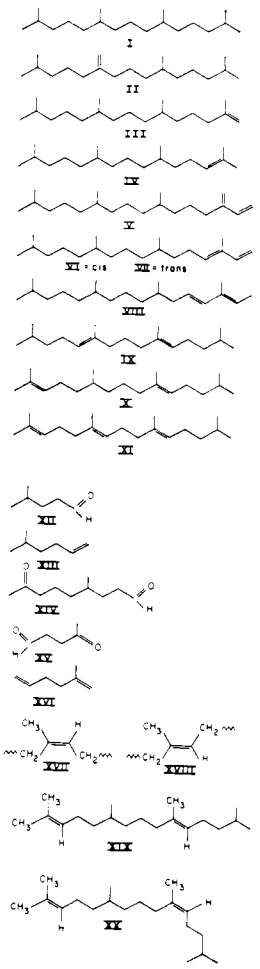
TABLE I: Gas Chromatographic Retention Indices.[a]

Compound	Apiezon L	RTV 502	Carbowax 20M	Emulphor O	FFAP	Porapak Q
I (pristane)	1692	1712	1675	1677	1669	
II		1730	1737		1732	
III		1731	1754		1748	
IV	1720	1743	1764	1749	1759	
V	1818	1841	1919		1925	
VI	1846	1863	1951	1928	1956	
VII	1866	1878	1979	1947	1984	
VIII		1901	2004		2011	
IX	1710		1796	1764	1797	
X	1732		1832	1796	1835	
XI	1736	1775	1885	1837	1887	
XII	732			953		
XV	819			1290		
Acetone				703		449

[a] Temperature programmed at 2–3°/min. Retention indices of unknowns and standards agreed to better than ±2 units.

Upon hydrogenation, all three compounds were converted into pristane (I), which was identified by its retention index on several polar and nonpolar columns. Thus, the identity of the carbon skeleton of the three olefins is established.



In the ultraviolet region of the spectrum, XI shows an absorption maximum at 210.5 nm, while IX and X have no maxima above 200 nm. Thus, conjugated double bonds are absent in all three olefins.

The infrared spectra of IX–XI are very similar. Aside from the expected strong methyl and methylene stretching absorption between 2800 and 3000 cm^{-1}, the most characteristic feature is the splitting of the asymmetric CH_3 bending band at 1370 cm^{-1} into a triplet. This feature, commonly found in isoprenoid structures, is ascribed to the presence of two geminal methyl groups, $CH(CH_3)_2$. The splitting is much more pronounced in IX, indicating a higher proportion of terminal methyl branching distant from ethylene unsaturation in IX than in X and XI.

Trisubstituted double bonds are indicated in IX–XI by a strong, broad band centered at 820–845 cm^{-1}. Unsaturation is also indicated by methine absorption at 3020–3040 cm^{-1}, but there is no suggestion of terminal vinyl groups or of disubstituted *cis* or *trans* double bonds.

Ozonides were prepared from very small (microgram) quantities of the olefins in a thin film, free of solvent, at the temperature of Dry Ice. They were studied by low-temperature pyrolysis in the gas chromatography inlet system under nitrogen. It is crucial that the decomposition be carried out at a low temperature. At 100° fragmentation occurs readily and the carbonyl derivatives are obtained in good yield and purity; at higher temperatures (150–200°), excessive scrambling leads to the production of a wide range of fragments. For the small samples this technique gave better results and easier isolation of the fragments than the conventional oxidation or reduction of the ozonides in solution.

Structures IX–XI were deduced from analysis of the pyrolytic fragments of the ozonides by gas chromatography, infrared spectra, mass spectrometry, and, in some cases, by synthesis. Ozonization of compound IX and subsequent pyrolysis–gas chromatography produces only two major fragments (mol wt 100 and 128) instead of the three expected for a diolefin. This suggests a symmetrical structure, producing identi-

TABLE II: Mass Spectrum of 4-Methyl-8-ketononanal.

m/e	Intensity	Assignment (Tentative)
170	0.32	M^+
169	0.21	$M^+ - 1$, loss of aldehyde proton
155	7	$M^+ - 15$, loss of CH_3
152	16	$M^+ - 18$, loss of H_2O
137	29	$M^+ - 33$, loss of CH_3 and H_2O
134	6	$M^+ - 36$, loss of 2 H_2O
127	11	β cleavage of aldehyde and loss of $CH_3C{=}O$
124	7	$M^+ - 46$, loss of H_2O and CO
113	10	Fragmentation of chain, see below, *m/e* 43
111	10	C_8 olefinic ion
109	56	$M^+ - 61$, loss of H_2O and $CH_3C{=}O$
99	5	Fragmentation of chain, see below, *m/e* 43
97	12	C_7 olefinic ion
95	53	$M^+ - 75$, loss of H_2O and CH_3COCH_2
85	15	Fragmentation of chain, see below, *m/e* 43
83	21	C_6 olefinic ion
81	22	$M^+ - 89$, loss of H_2O and $CH_3CO(CH_2)_2$
71	70	Fragmentation of chain, see below, *m/e* 43
69	44	C_5 olefinic ion
67	30	$M^+ - 103$, loss of H_2O and $CH_3CO(CH_2)_3$
58	78	Rearrangement peak of methyl ketone
57	30	Fragmentation of chain, see below, *m/e* 43
55	66	C_4 olefinic ion
43	100	Base peak, $CH_3C{=}O^+$ and β cleavage of aldehyde
41	52	C_3 olefinic ion $H_2C{=}CHCH_2^+$

Peaks of lower *m/e* obscured by gas chromatographic–mass spectrometric background

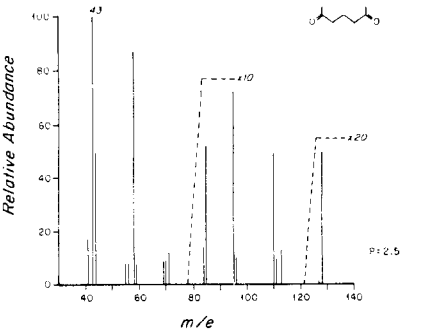


FIGURE 1: Mass spectrum of 2,6-heptanedione at 20 eV and 250° source temperature. *P* is the relative intensity of molecular ion.

cal fragments from two different segments of the pristane skeleton. The first of the pyrolysis products (mol wt 100) has a retention time and ΔI (Table I) suggesting a monofunctional C_6 carbonyl compound. The only structure compatible with the presence of a pristane skeleton is 4-methylpentanal (XII). This was synthesized through ozonolysis of 5-methyl-1-hexene (XIII); the mass spectrum and retention index of this product is identical with that of the fragment from IX. A symmetrical structure for IX, which leads to the formation of XII from either end of the pristane chain, should upon ozonolysis produce 2,6-heptanedione (mol wt 128) as the only other fragment. The mass spectrum of the second ozonolysis fragment from IX (Figure 1) is in excellent agreement with this assumption. Absence of an aldehyde function is indicated by the low intensity of the M − 1 peak and the absence of a M − 28 peak. The major peaks are due to the loss of CH_3 (*m/e* 113), H_2O (*m/e* 110), CH_3 and H_2O (*m/e* 95), and $CH_3C{=}O$ (*m/e* 85). The base peak (*m/e* 43) and rearrangement peak (*m/e* 58) are characteristic for methyl ketones. Thus, IX is 2,6,10,14-tetramethyl-5,10-pentadecadiene.

Pyrolysis–gas chromatography of the ozonide from X produces three major fragments; the molecular weights are 58, 100, and 170. The first compound to elute is acetone (mol wt 58), it is identified from its mass spectrum and the gas chromatographic retention time (Table I). The mass spectrum of the second peak is identical with that of 4-methylpentanal (XII) from IX and XIII. As monofunctional compounds, acetone and XII must be derived from either end of the pristane chain. Therefore, the remaining ozonolysis product should be the bifunctional C_{10} compound XIV, 4-methyl-8-ketononanal (mol wt 170). The mass spectrum of the corresponding product from X is in excellent agreement with this assumption (Table II). Thus, X is 2,6,10,14-tetramethyl-2,10-pentadecadiene.

Pyrolysis of the ozonide from XI produces three major fragments (mol wt 58, 100, and 100) instead of the four fragments expected from a triolefin. Again, this suggests that two identical fragments are produced from different sections of the chain. The mass spectrum and gas chromatographic retention index (Table I) identify the first compound as acetone. The second compound (mol wt 100) is 4-methylpentanal, its retention index and mass spectrum are identical with those of the C_6 aldehyde produced from 5-methyl-1-hexene, IX, and X. The third peak, also of mol wt 100, elutes much later than the second one; the ΔI is approximately twice that of 4-methylpentanal. This and the severe tailing on the Apiezon column suggest a difunctional compound. Its infrared spectrum shows clearly the aldehyde overtone bands at 2720 and 2820 cm^{-1}. The presence of the pristane skeleton and the identity of the other two pyrolysis fragments suggest that the third fragment is 4-ketopentanal (XV). This compound was synthesized from 2-methyl-1,5-hexadiene (XVI) by ozonolysis. The synthetic product and that from XI are identical in their gas chromatographic behavior (Table I) and in their mass spectra (Figure 2). The base peak at *m/e* 43 is characteristic for a methyl ketone ($CH_3C{=}O^+$) and for β cleavage of an aldehyde. The intense peak at *m/e* 57 characterizes the loss of $CH_3C{=}O$ and of CH_2CHO. The loss of 1, 18, and 28 (C_2H_4) mass units is typical of aldehydes, while the loss of 15 characterizes the methyl group adjacent to carbonyl. This combined evidence defines XI as 2,6,10,14-tetramethyl-2,6,10-pentadecatriene.

Proton magnetic resonance spectra of the above compounds

TABLE III: Proton Magnetic Resonance Spectral Parameters for Compounds IX, X, and XI.

Compd	Peak No.[a]	δ (ppm)	J^b (Hz)	No. of Protons		Assignment[d]
				Obsd[c]	Theor	
IX	1	0.86	5.4	10.8	12	4x $CH_3\dot{C}$–
	2	0.95				
	3	1.31	*e*	6.1	8	3x β>CH_2
	4	1.44				2x ⩾CH
	5	1.62	*f*	9.1	6	2x $CH_3\dot{C}$=
	6	1.69				
	7	1.92	*e*	6.3	8	4x α>CH_2
	8	2.03				
	9	2.15				
	10	5.04	*g*	1.9	2	2x H$\dot{C}$=$\dot{C}$–
X	1	0.85	5.4	8.5	9	3x $CH_3\dot{C}$–
	2	0.94				
	3	1.33	*e*	9.7	10	4x β,γ>CH_2
	4	1.44				2x ⩾CH
	5	1.61	*f*	9.1	9	3x $CH_3\dot{C}$=
	6	1.69				
	7	1.93	6.3[h]	6.8	6	3x α>CH_2
	8	2.03				
	9	2.14				
	10	2.25				
	11	4.85	6.6	2.0	2	2x H$\dot{C}$=$\dot{C}$–
	12	4.96				
	13	5.07				
XI	1	0.84	5.4	6.2	6	2x $CH_3\dot{C}$–
	2	0.93				
	3	1.12	*e*	5.5	3	1x β >CH_2
	4	1.25				1x ⩾CH
	5	1.36				
	6	1.59	*f*	9.0	12	4x $CH_3\dot{C}$=
	7	1.66				
	8	1.97	*e*	11.0	10	5x α>CH_2
	9	2.22				
	10	4.96	6.5	2.2	3	3x H$\dot{C}$=$\dot{C}$–
	11	5.06				
	12	5.17				

[a] All distinct peak maxima reported. [b] Nuclear spin–spin coupling constant where applicable. [c] Peak areas integrated by 1062 computer for compounds IX and X by an A-60A spectrometer for XI. [d] Assignments reported as the number of times a functional unit appears in the molecule. [e] Unresolved overlapping multiplets. [f] Separation of α-methyl proton peaks discussed in text. [g] Unresolved triplet. [h] Peak separation.

were obtained. The spectra are entirely consistent with structures IX–XI and amplify the available structural information somewhat. Spectral parameters and the assignment of peaks are given in Table III.

The rather broad resonance centered at approximately 5 ppm in the spectra of all three compounds is attributed to the olefinic proton of the expected $CH_2CH{=}C{<}^{C}_{C}$ grouping (Stehling and Bartz, 1966). In X (Figure 3) and XI this band is resolved into the triplet (coupling constant *ca.* 6.5 Hz) required by this structure.

Figure 4 serves to illustrate the main features of the aliphatic region of all three spectra.[1] The broad CH_2 bands which overlap the well-resolved methyl resonances lead to substantial errors in the estimation by integration of the number of protons of each type present in the molecule (Table III).

[1] The use of benzene rather than carbon tetrachloride as solvent in the determination of the aliphatic proton resonances in IX and X leads to possible complications due to solvent shifts. Such shifts, believed to arise from the donation of benzene electrons to an electron-deficient site in the solute molecule (Ronayne and Williams, 1967), would not, however, be particularly important in this case. The chemical shifts of the relatively narrow methyl peaks indicate that any solvent shift induced by benzene must be less than about 2 Hz.

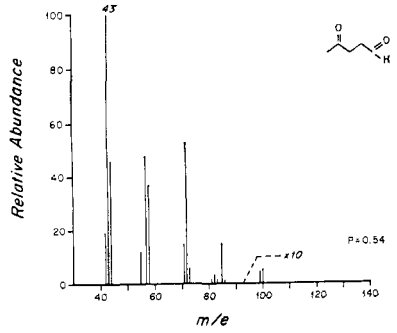


FIGURE 2: Mass spectrum of 4-ketopentanal at 20 eV and 250° source temperature.

Nevertheless, the high-field methyl doublet (peaks 1 and 2), arising from CH_3CH structures, clearly confirms four, three, and two methyl groups of this variety in compounds IX, X, and XI, respectively.

Complex multiplets at chemical shifts appropriate to β- and γ-methylene groups are observed in the spectrum of X (Figure 4) but they provide little information; the γ multiplet is largely obscured under the broad envelope of the β resonance. The spectra of IX and XI appear to be consistent with the absence of γ-CH_2 groups. In all three compounds, the multiplet arising from protons attached to a tertiary carbon atom is again obscured by the β band. The spectral band attributed to α-CH_2 groups in IX and XI allows little comment owing to the overlap of broad peaks in multiplets arising from protons in the situations $>C{=}C{<}^{H}_{CH_2CH_2}$ (4 and 6 protons in IX and XI, respectively) and $_{H}{>}C{=}C{<}^{C}_{CH_2CH_2}$ (4 protons in both). In X, however, the $>C{=}C{<}^{H}_{CH_2CH_2}$ type of α-CH_2 protons predominates (4 to 2) to a sufficient extent that the band should approximate a quartet. This occurs because the near equality of coupling constants (*ca.* 6 Hz) with the two β-CH_2 protons and the single olefinic proton approximates splitting by three equivalent nuclei (Stehling and Bartz, 1966). Figure 4 shows that this quartet is just resolved.

The two peaks assigned to methyl protons adjacent to a double bond are indicative of geometrical isomerism in which the olefinic proton is either *cis* or *trans* to the methyl group. In an investigation of *cis*- (XVII) and *trans*- (XVIII) polyisoprenes and mixtures of the two, Golub *et al.* (1962) showed that the *trans* methyl protons resonate at higher field than the *cis*. In the polyisoprenes, the chemical shifts for the *cis* and *trans* methyl protons are, respectively, 1.67 and 1.60 ppm. Feeney and Hemming (1967) have reported chemical shift differences of approximately 0.06 ppm between ω-terminal and internal α-methyl protons in the 100-MHz spectra of polyprenols dissolved in benzene. The expected additional separation of about 3.6 Hz is not resolved for the α-methyl protons in the 60-MHz spectrum of compound X; it is obscured in the line width of the peaks and by overlap of the methylene bands (Figure 4). On the basis of these studies, we attribute the resonances at 1.66–1.69 and 1.59–1.62 ppm to *cis* and *trans* groups, respectively, in IX–XI. In the case of X, which possesses only two geometrical isomers, it is

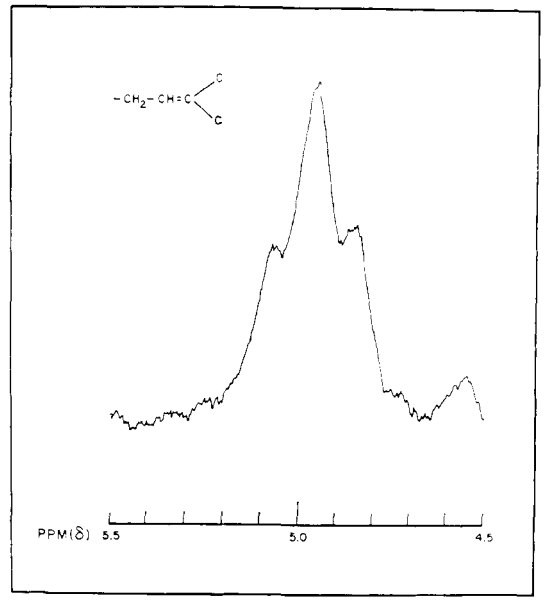


FIGURE 3: The olefinic region of the 60-MHz proton magnetic resonance spectrum from approximately 600 μg of X. The spectrum was scanned 4096 times with a sampling period of 10 msec/address by the 1024 channel, Fabri-Tek 1062 instrument computer.

further possible from the estimated relative areas of the two peaks to calculate the proportions of the two isomers present in the mixture. These are approximately 80% of isomer XIX to 20% of XX. The isomer ratio found can be regarded only as a rough estimate; for an accurate quantitative determination, complete separation of the α-methyl peaks at 100- or 220-MHz operational frequency would be required.

The gas chromatographic peak of X is broader than expected for a single isomer. Apparently partial resolution of the *cis* and *trans* isomer has been accomplished. To obtain a sample of maximum purity, only the material eluted near the peak maximum was trapped. Therefore, the ratio of *cis* to

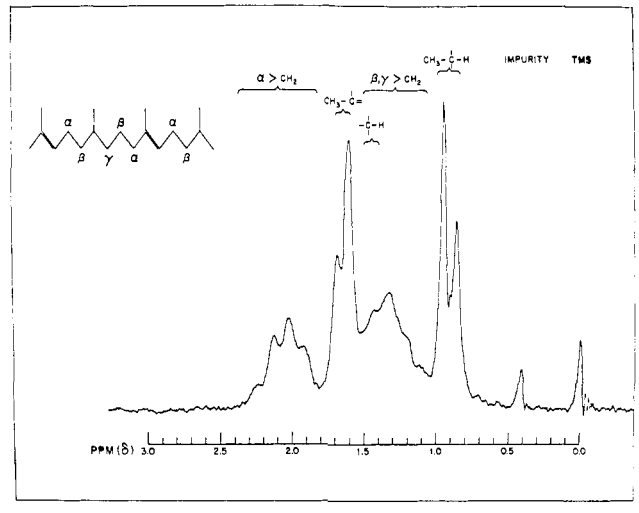


FIGURE 4: The aliphatic region of the 60-MHz proton magnetic resonance spectrum from approximately 600 μg of X. The spectrum was scanned 1024 times with a sampling period of 50 msec/address by the 1024 channel, Fabri-Tek 1062 instrument computer.

trans isomer reported here, while representative of the trapped sample, may not necessarily be representative of that in the original oil.

Gas chromatography suggests that small amounts of positional isomers, well separated from X and XI, may be present in the unsaturated hydrocarbon fraction in addition to the geometrical isomers. The complex chemistry involved in the derivation of the olefins from phytol (see Discussion) infers that it is not unreasonable to expect the presence of a complex mixture of isomers.

Experimental Procedure

Samples. The sources of *Calanus* species (Blumer *et al.*, 1964), of mixed zooplankton from the Gulf of Maine, and of basking shark lipids (Blumer, 1967) have been described earlier. Herring from the Gulf of Maine were obtained from the Bureau of Commercial Fisheries (West Boothbay Harbor, Maine). Pristane (2,6,10,14-tetramethylpentadecane, practical grade, Eastman Organic Chemicals) and Robuoy (Robeco Chemicals, Inc., New York) have a similar content of di- and triolefins. Isooctane (2,2,4-trimethylpentane, Phillips Petroleum Co., Bartlesville, Okl.) and benzene (Fisher Certified, ACS) were redistilled for use in chromatography. 5-Methyl-1-hexene and 2-methyl-1,5-hexadiene (Chemical Samples Co., Columbus, Ohio) were used without further purification.

Column chromatography on a dry bed of silica gel was followed by elution chromatography over the same adsorbent for further purification of the initial olefin concentrate. The adsorbent (Davison Silica Gel, Grade 922, Davison Chemical Co., Baltimore) was deactivated with 5% water to guard against isomerization of the olefins. The solvent was redistilled isooctane.

In a typical work-up, 300 ml of pristane was percolated over 300 ml of dry adsorbent. As much pristane as possible was displaced from the column by compressed nitrogen; elution was then started with 50-ml fractions of isooctane. The first two fractions were pure pristane, fractions 3–8 contained pristane and monoolefins. Diolefins, some triolefins, and a decreasing amount of pristane were eluted in fractions 9 and 10. Fraction 11 was largely triolefinic with pristane as the largest impurity.

Fractions 9 and 10 were again chromatographed on a 4-ml bed of silica gel in isooctane. After elution of the interstitial volume, 3 ml was collected. They contained pristane and monoolefins. The next 12 ml contained the diolefins together with some pristane, monoolefins, and a small amount of triolefins. The next 15 ml contained about 20% of XI; this was the major component of the last 30-ml fraction.

Fraction 11 of the initial chromatogram was purified in the same manner. Further chromatography yielded pure XI. Compounds IX and X were isolated by gas chromatography.

Gas Chromatography. The equipment consisted of Models 600 and 1200 Aerograph instruments with flame detectors, automatic attenuators, and 1-mV recorders. Columns were stainless steel ($^1/_8$ in. o.d. × 0.03 in. wall) operated at near-optimum plate efficiency at flow rates of approximately 11 cc of N_2/min. Packings used were Porapak Q (50–80 mesh) for the separation and identification of acetone, and Chromosorb G or W (acid washed, siliconized, and 80–100 mesh) for other separations. Liquid phases were Apiezon L, Carbowax 20M, FFAP (Varian Aerograph), Emulphor O (BASF, Ludwigshafen, Germany), and RTV 502 (Dow Corning; filler removed). Samples of the pure olefins were trapped in a glass capillary with etched inner wall to increase the surface area, from a 12-ft column packed with FFAP, 13.5% on Chromosorb W.

Mass spectra were obtained on a CEC 21-104 mass spectrometer interfaced with a gas chromatograph by means of a carrier gas separator (Blumer, 1968). Spectrometer conditions were: acceleration potential, 1600 V; magnetic scan, 1.5–16 A; anode current, 100 μA; ionization voltage, 20 eV; source temperature, 250°; and electron multiplier voltage, 130 V/stage.

Ultraviolet spectra were measured with a Cary Model 14 spectrophotometer in 1-cm quartz cells using redistilled and chromatographed isooctane.

Infrared spectra were obtained on a Perkin-Elmer, Model 337, spectrophotometer with beam condenser. Ozonolysis products were dissolved in chromatographed chloroform and measured in a 1-mm ultramicrocavity cell (Barnes Engineering Co., Stamford, Conn.). All other spectra were measured as smears between a 1 × 4 mm NaCl crystal and a larger NaCl plate, mounted on a holder with a 1 × 4 mm mask. The crystal was held to the plate by capillary adhesion.

Ozonolysis. Ozone was produced from oxygen, purified over Linde 5A Molecular Sieves, flowing through a 9-mm o.d. glass tube at 4 ml/sec. A discharge took place between a wire, inserted through a side arm and connected to a Tesla coil, and a grounded strip of aluminum foil (8 cm long) on the outside of the tube. No cooling was used. This apparatus produces 3 cc of ozone/min (KI titration).

The sample, spread on the etched outside of a glass tube (7 cm × 3 mm o.d.), was inserted into a larger tube, attached to the ozone generator, flushed with pure oxygen, and cooled to −70°. Ozone was then passed through the tube for 30 sec, the Tesla coil and gas flow were disconnected, and the sample was left in the ozone–oxygen mixture for 4 min at Dry Ice temperature and allowed to warm to room temperature a further 6 min. Ozone was removed by evacuation and the tube with the adhering ozonide was immediately inserted into the gas chromatographic injection port, which was held at 100°. The port was rapidly capped with nut and septum. The column was maintained at room temperature for 3 min and then temperature programmed for elution of the pyrolytic fragments of the ozonide.

Infrared spectra indicated that ozonization is incomplete at −70°. Complete conversion into the ozonides occurs only if the sample is permitted to warm to room temperature in the ozone atmosphere.

Proton magnetic resonance spectra were obtained on a Varian A-60A nuclear magnetic resonance spectrometer. Chemical shifts (δ) are reported in parts per million from tetramethylsilane.

The small sample size of IX and X required time averaging of the spectra. A Model 1062 instrument computer (1024 channel, Fabri-Tek Instruments, Inc.) was used in conjunction with the SD-2 digitizer and SW-3 field stabilizer and sweep control units. The trigger signal was generated by a Hewlett Packard 200 CD wide-range oscillator and monitored on a Hewlett Packard 521C electronic counter. The entire spectrum was first recorded at a 500-Hz sweep width to integrate the total peak area of the olefinic protons

relative to that of the aliphatic protons and to locate the resonance ranges. These were then scanned separately at narrower sweep widths to improve peak resolution and signal-to-noise enhancement. To measure the complete spectrum and that of the olefinic protons, the samples were dissolved in 99 mol % CCl_4 (Fisher Scientific Co.) stored for 24 hr over Linde 5A Molecular Sieves, with 1% tetramethylsilane added for triggering and as reference; a standard cylindrical nuclear magnetic resonance cell was used. Impurities in the CCl_4 necessitated the substitution of benzene as solvent to obtain a spectrum of the aliphatic protons. Tetramethylsilane (0.02–0.04%) was added as reference to 99 mol % benzene (Fisher Scientific Co.), which had been recrystallized four times. The samples were dissolved in 50 μl of this solvent in a semimicro cylindrical tube (Nuclear Magnetic Resonance Specialties); triggering in this case was effected by a benzene side band. By comparison of the peak area arising from the known percentage of tetramethylsilane present and the total aliphatic proton peak area, the quantities of IX and X used in the time-averaging experiments were estimated to be 350 and 600 μg, respectively.

Compound XI, isolated by column chromatography in sufficient quantities to permit detection on a single scan of the spectrum, was dissolved in Spectrograde CCl_4 containing a small amount of tetramethylsilane and its spectrum was measured in a standard cylindrical nuclear magnetic resonance cell.

Discussion

This work completes the structural identification of the principal phytol-derived hydrocarbons which enter the marine food chain from *Calanus*. We feel that this detailed work has been necessary and should be followed by complete stereochemical analysis, because of the great importance of *Calanus* in the marine food chain and because of the persistence of these hydrocarbons in the marine environment. A further investigation of the stereochemical relationships among the hydrocarbons and between them and their presumed precursors would be of interest and might provide positive information about their origin. The extremely low concentration of these compounds in marine organisms precludes such work at the present time.

We believe that the olefins II–XI are all derived from phytol in the diet of the zooplankton, but that at least two different mechanisms are involved in the conversion. Phytol is easily dehydrated to neophytadiene (V). The isomeric phytadienes VI–VIII can be produced at the same time or independently by isomerization of V. Dehydration of phytol takes place under the influence of mild catalysts such as activated chromatographic adsorbents (Johnstone and Quan, 1963). It is not surprising that it may also occur in the digestive system of planktonic animals.

Base-catalyzed dehydration of phytol also leads to C_{19} monoolefins, in surprisingly high yields, according to Johnstone and Quan (1963), 14–49%, depending upon the conditions of the reaction. The principal product appears to be norphytene (III) but Johnstone suggests that it is accompanied by a product in which the double bond has migrated farther toward the center of the chain. It can be expected that isomerization of III will easily lead to IV, the most abundant C_{19} olefin of *Calanus*.

Catalytic dehydration of phytol and subsequent isomerization of the olefins cannot alone account for the formation of the remaining olefins. In these, the double bonds are present in numbers and at positions not likely to be reached through isomerization. Different biochemical mechanisms may be involved in the formation of compounds II and IX–XI; for instance, these olefins might be formed from fatty acid precursors through decarboxylation. Marine organisms like cod (Iverson, 1967) and *Calanus*, basking shark, and tuna (Blumer, 1967) contain saturated, mono- and polyolefinic isoprenoid acids; all carbon numbers from C_{15} to C_{22} are represented. It would be interesting to study the structures of the unsaturated marine isoprenoid acids from the point of view of their relationship to the olefins.

Isoprenoid hydrocarbons are refractory and are not altered in the digestive tract of carnivorous copepods and in marine fishes which feed on *Calanus* (Blumer *et al.*, 1964). Thus, the absolute concentrations and the concentration ratios of I–XI in the liver of a basking shark were found to be identical with those of the copepods in the stomach of the shark and in the surrounding water mass (Blumer, 1967). We have suggested that in their persistence the isoprenoid hydrocarbons, together with the great variability of hydrocarbon composition between different species of marine zooplankton, should provide characteristic and long-lived markers for the study of dynamic processes in the marine food chain. Thus, they may serve as biochemical integrators for the amount of a certain food ingested by an animal, or as markers to follow the migration of marine fishes. We have recently demonstrated that the liver of herring taken in the Gulf of Maine contains hydrocarbons of the same structure and type distribution as *Calanus*, the principal zooplankton species in that region. Herring, taken off New Jersey, on the other hand, have a very different hydrocarbon composition. This suggests that their principal food source differs from that of the animals in the Gulf of Maine. Further studies in this direction are promising and needed; the data presented here and similar data on other species of marine plankton will form the background for proper interpretation of the data.

The pollution of the sea by petroleum hydrocarbons increases at a rapid pace. It is important to know that even before man's arrival on the earth, hydrocarbons were not foreign to the marine environment. If hydrocarbons are isolated from a marine source, they need not necessarily be derived from pollution. Fortunately, natural hydrocarbons and pollutants are sufficiently different in molecular weight and type distribution to be easily distinguished from each other. Olefinic hydrocarbons of the type described here are not present in petroleum; therefore they should provide ideal markers for natural marine hydrocarbons. Aromatic hydrocarbons, on the other hand, are major components of petroleum but have not been isolated from the planktonic hydrocarbon fraction (Blumer, 1969).

Saturated isoprenoid hydrocarbons, including pristane and phytane, are common and abundant in geological samples. However, we do not believe that the olefinic hydrocarbons from marine lipids are major contributors to organic geochemicals; they are after all very minor constituents, relative to pristane and its even more abundant precursor, phytol. Also, numerous other mechanisms are available for the formation of the fossil isoprenoids. Recent sediments have been shown to contain pristane, presumably of biochemical

origin, but neither phytane nor olefinic isoprenoids have been isolated in detectable quantities (Blumer and Snyder, 1965). We do not, however, exclude the possibility that phytadienes and C_{19} olefins, formed *in situ* by catalytic dehydration of phytol play a role as intermediates in the formation of fossil phytane and, *via* degradation at greater depth and temperature, of its lower homologs.

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The Derivatization of Cross-Linked Polyacrylamide Beads. Controlled Introduction of Functional Groups for the Preparation of Special-Purpose, Biochemical Adsorbents*

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ABSTRACT: Methods are described for the chemical modification of preformed, cross-linked polyacrylamide beads for the purpose of introducing any of a wide variety of functional groups to a predetermined level. Examples are given of the conversion of commercially available polyacrylamide beads into a variety of ion-exchange media, of differing titration range and capacity, capable of binding protein molecules. The use of chemically reactive bead derivatives for the covalent binding of proteins is seen in the examples of solid-phase coupling of trypsin and bovine serum albumin to beads. The reversible reaction of rabbit antibody with bovine serum albumin antigen covalently coupled to acrylamide beads is described.

Modified hydrophilic polymers of natural origin such as cellulose and cross-linked dextran have been used extensively as carriers for biochemical separations based on ion exchange (Sober *et al.*, 1965) or the covalent bonding of biochemically active molecules, either large (Campbell *et al.*, 1951) or small (Cuatrecasas *et al.*, 1968). These natural polymers are polysaccharide in nature and their chemical stability and reactivity is limited by the sugar groups of which they are composed. In the case of cellulose derivatives, furthermore, the complex physical structure of the starting material may be responsible for nonuniform substitution in the product.

Polyacrylamide beads as support medium offer the advantage of enhanced chemical stability by virtue of their polyethylene backbone structure, coupled with a statistically uniform physical state and porosity resulting from their formation as cross-linked synthetic polymers. The beads are readily available commercially in spherical form, in pregraded sizes and porosities, permitting the penetration of macromolecules up to molecular weights of approximately one-half million. It seems probable that wide use would be made of preformed polyacrylamide beads as carrier matrix for biochemical separations if methods were available for the controlled introduction of chemical groups suitable for ion-exchange or covalent formation. Methods fulfilling these requirements are described below.

Polyacrylamide beads contain a hydrocarbon skeleton to which is attached carboxamide side groups. These groups are chemically stable and resistant to hydrolysis in the pH range between 1 and 10. The amide ammonia nitrogen, however, is readily replaced by certain other nitrogen compounds per-

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