Studies on the Minor Constituents of Whale Oils—I.
Identification of 2, 6, 10, 14-Tetramethylpentadecane
and 2, 6, 10, 14-Tetramethyl-2-pentadecene
in Sperm Blubber Oil

Yoshihiko Sano*
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A saturated polyisoprenoid hydrocarbon, pristane, has previously been shown to
be a probable constituent of sperm whale oil, and unfortunately C_{18}H_{36} has been
quoted as its molecular formula for a long time. The structure of pristane, isolated
from the liver oil of basking shark, has been determined as 2, 6, 10, 14-tetramethyl-
pentadecane (C_{19}H_{40}) by Sorensen et al. and by Sugiya et al. Recently, three
isomeric C_{19} mono-olefins (zamene) as well as pristane have been isolated from mixed
zooplankton of the Gulf of Maine and from the liver oils of various marine fishes
and mammals, including sperm whale, and the structures have been elucidated briefly.

This paper describes the separation and identification of the multibranched-chain
hydrocarbons with the carbon skeleton of pristane or presumable phytane (3, 7, 11, 15-
tetramethylhexadecane) from sperm blubber oil.

Experimental Procedures and Results

Materials and Methods. The sample sperm blubber oil (acid value, 1.13; iodine
value (Wijs), 78.4; unsaponifiable matter, 39.7%) was obtained through the courtesy
of Mr. T. Koga, Taiyo Fisheries Co., Ltd., and the oil was prepared by steam-
rendering of the blubber of an adult sperm whale, Physeter macrocephalus, approximately
15 m in length, caught in the waters off Akkeshi, Hokkaido, Japan.

All solvents used for chromatography were redistilled with a glass apparatus.

Analytical thin-layer chromatography (TLC) was carried out on plates coated
with Silica Gel G (according to Stahl) or 5% silver nitrate-impregnated Silica Gel G,
as described previously. n-Hexane-diethyl ether (90 : 10, v/v) was used as the
developing solvent. Spots on the analytical plates were visualized by H_{2}SO_{4} charring
or iodine vapor.

Gas-liquid chromatographic (GLC) analyses of hydrocarbons were performed on a
3 mm i. d. × 1.85 m stainless steel tubing packed with 10% polyethylene glycol adipate
(PEGA) on Diabase B (Kotaki Seisakusho, Ltd., 80～100 mesh) in a Shimadzu gas
chromatograph, Model GC-1C, equipped with a hydrogen flame ionization detector,
at temperatures from 180°C to 210°C. The operating conditions are described in Fig. 2.

* Research Laboratory, Miyoshi Oil & Fat Co., Ltd., 4-chome, Horikiri, Katsushika-ku, Tokyo
(佐野吉彦, ミヨシ油脂(株)研究部).
The peaks were identified by comparison with standard mixtures and by the equivalent chain length (ECL) calculated from a semi-logarithmic plot of retention times versus chain length.

Infrared spectra were recorded by a twin-beam spectrophotometer, Model IR-S (Japan Spectroscopic Co., Ltd.), by thin films between rock-salt disks.

Nuclear magnetic resonance (NMR) spectra were obtained with a Varian A-60 spectrometer equipped with a Varian NMR integrator on deuterochloroform solutions containing tetramethylsilane (TMS) as internal standard.

Mass spectra were run at 70 eV using a Hitachi Model RMU-6D instrument under the operating conditions described in Fig. 4.

Hydrogenation was done in \( n \)-hexane at room temperatures with a micro-hydrogenator over a platinum oxide catalyst.

**Separation of the hydrocarbons from sperm blubber oil.** The sample oil (120 g) was dissolved in 60 ml of \( n \)-hexane, neutralized with a slight excess of 20% potassium hydroxide solution, and purified by passing through a silicic acid column, as previously
The refined oil (110.5 g) was subsequently chromatographed on a large column (8 × 50 cm) packed with 2 pounds of silicic acid (a Mallinckrodt product, 100 mesh). Elution with n-hexane (total 2,000 ml) yielded a hydrocarbon fraction (292 mg, see Fig. 1, fraction A), and further elution with n-hexane containing the increasing amounts of diethyl ether gave wax and triglyceride fractions.

Fractionation of the hydrocarbon fraction by argentation-silicic acid column chromatography. Fractionation of the mixed hydrocarbons (290 mg) above-mentioned was accomplished by column chromatography on silicic acid (5.0 g) impregnated with 10% silver nitrate, as previously reported. Following elution of the major fraction (F-1, 280 mg) with n-hexane (50 ml), two further fractions (F-2 (1 mg) with n-hexane (50 ml) and F-3 (4 mg) with 10% diethyl ether in n-hexane (50 ml)) were obtained.

Identification of 2, 6, 10, 14-tetramethylpentadecane in sperm blubber oil. Fig. 2 represents a gas chromatogram of the fraction F-1 on the PEGA column. The major peak (99%), having an ECL value of 16.7, was enhanced when this fraction was coinjected with an authentic sample of 2, 6, 10, 14-tetramethylpentadecane sythesized from phytol, as described previously.

The infrared spectrum of the fraction F-1 was identical with that for an authentic sample of 2, 6, 10, 14-tetramethylpentadecane and was in complete agreement with published spectra for this compound. A strong band at 735 cm⁻¹ (unaccompanied by a prominent shoulder at 727 cm⁻¹) resulting from the methylene rocking vibration of the \( R-(\text{CH}_2)_n-R \) grouping proved to be most significant in establishing the regular isoprene chain sequence. The strong band appearing at 1170 cm⁻¹ with a shoulder at 1150 to 1155 cm⁻¹ is attributed to the skeletal vibrations of the terminal groups, while the C-H deformation modes of these groups give rise to two closely spaced bands near 1380 cm⁻¹, indicating the presence of an isopropyl group.

Fig. 4 shows the mass spectrum of the fraction F-1. The molecular ion peak was at m/e 268, thus giving the formula \( \text{C}_{19}\text{H}_{40} \). A prominent alkyl fragment peak occurred at m/e 183, which corresponds to \( \text{C}_{13}\text{H}_{27}^+ \). In addition, the points of branching were easily distinguishable. The peak at m/e 253 (M−15) indicated methyl branching in the molecule. Alkenyl ion peaks (\( \text{C}_n\text{H}_{2n−1}^+ \)) and olefin ion peaks (\( \text{C}_n\text{H}_{2n}^+ \)) were also found on the spectrum. The olefin ions (at m/e 112 and 182), which are not found on the spectrum of normal paraffins, appeared only at masses corresponding to branching points (\( \text{C}_8 \) and \( \text{C}_{10} \)). These data were in agreement with the mass spectrum of 2, 6, 10, 14-tetramethylpentadecane published by Bendoraitis et al.

Additional confirmation of the extensive branching, indicated by both infrared and mass spectra, was obtained by means of the NMR spectrum. The spectrum in Fig. 5 showed a single methylene proton at \( \delta 1.2 \) and two strong methyl proton signals. The methyl proton resonance at \( \delta 0.85 \) was split into a doublet with a separation of 5.7 cps, presumably the effect of tertiary proton coupling. An expected tertiary proton resonance on the low field tailing of the methylene proton resonance
was detected. The number of methyl groups per molecule calculated from area under the methyl proton resonance and a molecular weight of 268 (40 protons) turned out to be 6.

From the foregoing gas-liquid chromatographic analysis and infrared, mass and NMR spectrometric studies, a major component in the fraction F-1 was identified as 2, 6, 10, 14-tetramethylpentadecane (pristane).

**Gas-liquid chromatographic analyses of the fraction F-2.** The gas chromatogram of the fraction F-2 showed five main peaks, as given in Fig. 6. A major peak (P) had

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**Fig. 5.** NMR spectrum of the fraction F-1.
Operation conditions:
Solvent: CDCl₃; Filter bandwidth: 1 cps;
R. F. Field: 0.4 mG; Sweep time: 500 sec.;
Sweep width: 500 cps; Spectrum amp.: 2.0 x 10.

**Fig. 6.** Gas-liquid chromatograms of the fraction F-2 and its hydrogenated product (dotted line).
GLC conditions are the same as given in Fig. 2.
the same retention time as 2, 6, 10, 14-tetramethyl-2-pentadecene synthesized from phytol, as described previously. The formation of 2, 6, 10, 14-tetramethylpentadecane from the component, on hydrogenation, confirmed its structure.

The structures of the other minor materials in this fraction remain unknown. But it is assumed that most of these constituents are an unsaturated branched-chain hydrocarbon, judging from the ECL values of the hydrogenated product.

**Gas-liquid chromatographic analyses of the fraction F-3.** On gas-liquid chromatography, in the fraction F-3 appeared a major peak having an ECL value of 19.8, as illustrated in Fig. 7, while that of the main peak in its hydrogenated product was 17.8. This value was similar to that (17.86) of 3, 7, 11, 15-tetramethylhexadecane. Therefore, the major constituent (Q) in this fraction may be 1,3-phytadiene with the trans structure, judging from the data on zooplankton published by Blumer and Thomas. However, this component still remains to be identified completely. Further work will be required to identify the constituents of this fraction.

**Discussion**

Pristane was first discovered in 1917 by Tsujimoto in the liver oil of basking shark and its presence was subsequently established in a large variety of marine organisms, such as several species of shark, fishes (herring, sardine, and bonito), zooplankton, sei whale, and sperm whale, and further in wool wax, petroleum, pre-Cambrian sediment and so on. Thus, the compound is presently shown to be widely distributed in minor amounts except shark liver oils.

Recently, Mori et al. reported that the content of hydrocarbon in a sample of sperm whale oil was approximately 0.16% of the total weight of the oil by means of column chromatography. As for the sperm blubber oil used in this investigation, it was observed that the sample oil contained 0.24% of hydrocarbons, more than 98% of which were pristane. So, it should be noted that the most prominent component of hydrocarbons from sperm blubber oil is 2, 6, 10, 14-tetramethylpentadecane.

Blumer and Thomas reported briefly that three isomeric C₁₉ olefins with the
carbon skeleton of pristane, namely 2, 6, 14-trimethyl-10-methylene pentadecene (A),
2, 6, 10, 14-tetramethyl-1-pentadecene (B), and 2, 6, 10, 14-tetramethyl-2-pentadecene
(C), have been isolated from mixed zooplankton, and from the liver oils of fishes,
sharks, and sperm whale (*Physeter catodon*); and that structure C was the predominant
one in all cases, exceeding structures A and B by an approximate factor of ten in
the zooplankton extracts and the liver oil of the basking shark. This predominance
of structure C was observed for the sperm blubber oil studied here. Unfortunately,
we have been unable to detect other isomers of pristene (Zamene) in the fraction
F-2 (see Fig. 6).

Very little is known about the occurrence of phytadienes in sperm oil. However,
the major component in the fraction F-3 (see Fig. 7) may be related to phytadienes
detected in zooplankton on the basis of its ECL value of 19.8 (19.79 ± 1.14 for trans-1,3-
phytadiene) and that (17.8, 17.86 ± 1.14 for phytane) of its hydrogenated product.

As regards the origin of multibranched-chain hydrocarbons found in the sperm
blubber oil, it is assumed that these hydrocarbons are derived from phytol, as
previously suggested. These hydrocarbons have an unusually low density and
melting point, and may therefore serve in keeping the stored lipids of sperm whale
as well as nekton in the liquid state.

Other many materials present in trace amounts have not been characterized. We
wish to report in the near future the structure of these minute constituents of sperm
blubber oil.

**Summary**

A multibranched-chain hydrocarbon, having an ECL value of 16.7 on the PEGA
column, was isolated in a purity of 99% by column chromatography on silicic acid
and on silicic acid impregnated with 10% silver nitrate from the blubber oil of sperm
whale, *Physeter macrocephalus*. This hydrocarbon was determined as 2, 6, 10, 14-
tetramethylpentadecane (pristane) by means of gas-liquid chromatography (GLC),
infrared analysis, high resolution nuclear magnetic resonance and mass spectrometry.

In addition, a component, having an ECL value of 17.4, in the unsaturated
branched-chain hydrocarbon fraction was tentatively identified by GLC as 2, 6, 10, 14-
tetramethyl-2-pentadecene, and a presumable existence of phytadiene was observed.

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