

Studies Related to Pristane

I. The Unsaponifiable Matter of the Liver Oil of the Basking Shark

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Mitsumaru Tsujimoto¹ discovered in 1917 in the unsaponifiable matter of the liver oil of the basking shark (*Selache maxima* Gunnerus) a saturated hydrocarbon which later was named pristane². Tsujimoto established the aliphatic nature of this hydrocarbon; as regards the molecular formula, Tsujimoto found it difficult to decide between $C_{17}H_{36}$, $C_{18}H_{38}$ and $C_{19}H_{40}$, we quote: »It is not safe to say that the substance was a single chemical compound, but judging from its boiling point and molecular weight, it appears to be very probable that it consisted mainly of $C_{18}H_{38}$ or an octadecane. But as the normal octadecane is a solid at the ordinary temperatures, the substance must be at least an isooctadecane.»

Later Tsujimoto's pupil Toyama² proved the occurrence of pristane in some 5 further Elasmobranch-liver oils, but added very little to the chemistry of pristane. Unfortunately pristane in literature²⁻⁴ is mostly quoted as isooctadecane, regardless of Tsujimoto's cautious statement. Probably the high m. p., $-11^{\circ}C$, stated by Toyama and Tsuchiya⁵ has contributed to this disregarding of the statement of Tsujimoto⁶, that purified pristane does not solidify at the temperature of solid carbon dioxide. This very low m. p. is quite incompatible with the structure of pristane as iso-octadecane = 2-methylheptadecane, the m. p. of which is $+5^{\circ}C$.

Our interest was attracted to pristane as a result of biosynthetic discussions. As far as known, the fatty acids of the basking-shark liver oil are normal unbranched fatty acids. The unsaponifiable matter of the oil consists for the most part *cf.* 7-9 of the unsaturated triterpene hydrocarbon squalene $C_{30}H_{50}$. We would find it most remarkable if the basking-shark was capable of the normal fat synthesis, of the synthesis of terpenoid compounds as well as of the intermediate type of methylsubstituted chain compounds primarily discovered by Anderson and Spielman in the tubercelostearic acid¹⁰; in the latest years

increased through Weitkamp's excellent studies of the acidic constituents of de-gras¹¹. So we found it worth while to prove the indicated constitution of pristane.

Chem. Eng. K. Toft-Eriksen⁹ informed us that he once, on fractional distillation of one of his samples of basking-shark liver oil, observed crystals in the cooler at the end of the pristane fraction. As the temperature of the cooling water was greater than + 5°, the possibility, that the crystals were pure iso-octadecane, could at once be excluded. Some small samples of the oils investigated by Toft-Eriksen were at hand and we ascertained that two of them solidified at — 5°. So we decided to elucidate the nature of these crystals.

By carefully fractionating some kg of basking-shark oil in a 70 cm column we found that the oil regularly gives a broad fraction between pristane and squalene; the raw middle-fraction solidified by room temperature at once to a crystal jelly. By filtering and recrystallizing as described in the experimental part, this middle-fraction was divided into some pristane and squalene, and the three fatty alcohols cetyl-, stearyl- and oleyl alcohol.

The two saturated alcohols cetyl- and stearyl alcohol have a low solubility in pristane below room temperature and as they crystallize in great, but very thin leaflets which pile up on one another to a very voluminous framework even contaminations of 1 % of these saturated alcohols in pristane will bring the solution to solidify at 0°.

Besides the mentioned compounds, the basking shark liveroils as well as all liver oils of higher animals, contain cholesterol. The quantity was estimated, through precipitation with digitonin, to 0.4 % of the unsaponifiable matter. As regards the unsaponifiable matter of our great sample pure basking-shark oil, for which we are very much indebted to Chem. Eng. Alf Brautaset, Jahres Kjemiske Fabrikker A/S, Sandefjord, we may state the following approximate composition:

Cholesterol	0.4 %
Pristane	14 %
Squalene	84 %
Cetyl alcohol	0.6 %
Stearyl alcohol	0.4 %
Oleyl alcohol	0.3 %

The three fatty alcohols have before been found a few times in fish oils¹² each of them about 5 times, distributed in many classes of fish. Mostly they are found in small quantities and in all they are much more scarce than the glycerol-ether-alcohols, chimyl, batyl and selachyl alcohol. These alcohols very common among fishes are absent in basking-shark liver oil, as the oils give no isopropyl iodide after Zeissel Fanto.

To eliminate the alcohols the pristane fractions were purified over metallic sodium. Redistilled once more, pristane is a colourless liquid, which does not solidify at -80° . As shown by Tsujimoto¹², such refined pristane is not pure, but contains various small amounts of an unsaturated hydrocarbon, called zamene. Zamene can be quantitatively withdrawn through a few treatments with 100 % H_2SO_4 . In this way refined pristane has physical constants in excellent agreement with the Japanese authors. With all constants referred to 20° and corrections of some evident misprints we have

	d_4^{20}	n_D^{20}
Tsujimoto 1917 ¹	0.78437	1.4398
Toyama 1923 ²	0.7835	1.4390
Present investigation	0.78267	1.43848

From a biochemical point of view the simplest assumption would be that pristane is a saturated hydrocarbon of terpenoid structure. But as partially degraded terpenoids are much more rare than compounds with integral numbers of C_5 , much importance is attached to the molecular weight of pristane. Professor dr. Endre Berner on our request most amiably carried out a precision determination of the molecular weight of our purest specimen (cryoscopic in benzene). As comparison compound we handed to Berner a sample of 2,6,11,15-tetramethyl-hexadecane = »Crocetane«, synthesized for this purpose by Chem. Eng. Hugo Holtermann through hydrogenation of the previously unknown diterpene 2,6,11,15-tetramethyl-hexadeca-tetraene-2,6,10,14 = »Digeranyl«. The details of this synthesis will follow in another publication of this series. For pristane Berner found mol. wt. 269, and 273, for »Digeranyl« 282, against calculated for $C_{20}H_{42} = 282.4$, $C_{19}H_{40} = 268.4$ and $C_{18}H_{38} = 254.4$.

So we must conclude that pristane is not an octadecane at all, but at least a nona-decane. It must be added that Tsujimoto gave two determinations *viz.* 265, 252; Toyama measurements on not less than nine samples; his values ranges from 259 to 270, mean 264.1 ± 1.2 . The measurements of the Japanese authors themselves scarcely justify their own conclusion. As experimental errors systematically tend to lower the molecular weight determinations, already their original determinations suggest a molecular formula not lower than $C_{19}H_{40}$. However, we agree with Tsujimoto in his statement of the uncertainty of the homogeneity of the pristane hydrocarbon and so, in spite of the excellent agreement of professor Berners measurements for pristane with $C_{19}H_{40}$ we do not venture to exclude a molecular formula of $C_{20}H_{42}$.

Of saturated terpenoid hydrocarbons $C_{20}H_{42}$ Willstätter, Mayer and Hüni¹⁴ has prepared »Phytane« (2,6,10,14-tetramethyl-hexadecane) from

phytol; Karrer and Golde¹⁵ has transferred the carotinoid pigment Crocetin in the isomeric above mentioned hydrocarbon »Crocetane«. From geraniol the same hydrocarbon has been synthesized by Fischer and Löwenberg¹⁶. As will be seen from Table 1, the physical constants of these two preparations differ appreciably. The data of Willstätter for »Phytane« and of Fischer and Löwenberg for »Crocetane« come very close to the physical constants of pristane and so augment the possibility that pristane is a saturated diterpene. As, however, there can be no doubt about the constitution of crocetin nor about the »Crocetane« of Karrer we found it necessary once again to synthesize this hydrocarbon.

Table 1. Physical constants of the saturated aliphatic diterpenes, $C_{20}H_{42}$.

Author	d	n
Phytane (Willstätter)	$(d_4^0 = 0.803)$	—
	$d_4^{20} = 0.78936$	—
Crocetane (Karrer)	$(d_4^{19.8} = 0.8027)$	$(n_D^{19.8} = 1.44937)$
	$d_4^{20} = 0.8025$	$n_D^{20} = 1.4492$
Crocetane (Fischer)	$(d_4^{25} = 0.7853)$	$(n_D^{25} = 1.4382)$
	$d_4^{20} = 0.7887$	$n_D^{20} = 1.4402$
Crocetane (present investigation)	$d_4^{20} = 0.78584$	$n_D^{20} = 1.44055$

The synthesis was carried out from citral via tetrahydrogeraniol and tetrahydrogeranyl bromide along much the same lines as by Fischer and Löwenberg. In Table 1, all data have been quoted from the originals and recalculated to 20°. As will be seen the data from our preparation of »Crocetane« is in accordance with those of Fischer. As to the deviating data of Karrers Crocetane, we shall return to them in a later communication.

EXPERIMENTAL

The crystalline alcohols

The unsaponifiable matter of basking-shark oil was distilled in batches of ca 550 g through a 70 cm column whose temperature could be regulated electrically from 75—350°. All distillations were carried out between 0.05—0.1 mm, the temperature of the column was regulated some 35° lower than the oil bath temperature. The fractions below 110° contain pristane contaminated with small amounts of some oxidation product of squalene with characteristic lemon odour, the fractions above 185° contain only squalene. There was no definite boiling fractions between pristane and squalene; arbitrarily, how-

ever, we separated the intermediary distillate in the fractions b. p. 110—145° and 145—185°. Fraction 110—145° solidified only when cooled, by the distillation of the fraction 145—185° the recipient and the connecting pipes had to be gently warmed to avoid tightening through crystallization. 550 g unsaponifiable matter gave approximately 55 g < 110°, 27 g between 110—145° and 38 g between 145—185°. The two last fractions were cooled down to — 17° and after some time each of them brought on porous porcelain at — 17°. In all 3,280 g unsaponifiable matter were in this way converted to 16 g raw crystals from the fraction 110—145° and 28 g from the fraction 145—185°. Both crystal fractions were recrystallized from acetone solution at 0° C, the second one then from alcohol at 0°. The m. p. showed the following displacement:

	Fraction I	Fraction II.
	M. p.	M. p.
From acetone 1	8 g 44.5—47°	9 g 47 —49°
» » 2	6 » 48 —49°	6 » 54.5—56°
» » 3	4 » 48 —49°	4 » 55 —56.5°
From alcohol 4		1.5 » 56 —57°
» » 5		1 » 56 —57°

Through fractional distillation of spermaceti and repeated recrystallization there were prepared pure cetyl and stearyl alcohol. Cetyl alcohol from spermaceti m. p. 48—49°, mixed m. p. 48—49°.

Stearyl alcohol from spermaceti m. p. 56.5—57.5°, mixed m. p. 56—57°.

Fraction I, 3.

$C_{16}H_{34}O$ (242.3) Calc. C 79.25 H 14.14 Mol. wt. 242.3
 Found » 78.9, 79.2 » 14.1, 14.35 » » 242.5, 241

Fraction II, 5.

$C_{18}H_{38}O$ (270.3) Calc. C 79.95 H 14.13 Mol. wt. 270.3
 Found » 79.1, 79.7 » 13.85, 14.05 » » 268, 270

According to the procedure of Årtun¹⁷ the cetyl alcohol as well as the stearyl alcohol from spermaceti and basking-shark liver oil was transformed into the 3,5-dinitro-benzoates.

3,5-dinitro-benzoate of cetyl alcohol from spermaceti m. p. 66—67°
 » » » » » » » basking-shark m. p. 66—67°
 » » » » stearyl alcohol from spermaceti m. p. 74—75°
 » » » » » » » basking shark m. p. 74—75°

Mixed m. p. with each pair no depression.

Liquid alcohols

From all crystallizations and from the porous plates the mother liquors were collected and as much saturated alcohols as possible removed through crystallizations at low temperatures. The physical constants indicated that the liquid residues of Fraction I mainly consisted of pristane, that from II of squalene. Through »Entmischung» between petrol ether and methanol of 95, 92.5 and 90 % the remaining alcohols were separated

from the hydrocarbons, which constituted from 5/6 to 19/20 of the liquids. The hypophasic part showed

$$n_D^{20} = 1.4770, \text{ iodine number } 102, \text{ and a m. p. about } 0^\circ.$$

Oleyl alcohol.

$$\begin{array}{l} C_{18}H_{36}O \text{ (268.3)} \quad \text{Calc. C } 80.51 \quad \text{H } 13.53 \quad \text{M. p. } +2-3^\circ \\ \text{Found } \ast 79.9, 79.3 \ast \quad 13.0, 13.67 \quad \ast \quad 0^\circ \end{array}$$

The data are in accordance with a slightly contaminated oleyl alcohol. The most suitable manner to prove the occurrence of oleyl alcohol is the hydrogenation to stearyl alcohol. This hydrogenation was carried out with Pt-silicagel at room temperature, the theoretical amount H_2 was taken up in 8 hours. The residue after hydrogenation was solid m. p. 56—57°, constant after recrystallization.

«Crocetane» from citral (stud. chem. Per Koch-Christensen)

Citral was carefully fractionated in a vacuum, and at once hydrogenated with Raney-nickel by 90° under 50—60 atm. H_2 (compare Palfray¹⁸). 3,7-dimethyloctanol-1 distilled at 114—116° at 21 mm and showed $n_D^{20} = 1.4351$; Palfray gives 106°/12 mm and $n_D^{20} = 1.4367$. 3,7-dimethyloctanol-1 were converted to the corresponding bromide according to¹⁹ B. p. 108—111/24 mm; $n_D^{19} = 1.4538$.

Longinow and Margoliss²⁰ give $n_D^{20} = 1.4534$. 63 g 3,7-dimethyloctylbromide, 16 g metallic potassium and 250 g anhydrous benzene were boiled with reflux for 20 hours, and the reaction product fractionated. The fraction 144—155/2 mm was distilled over Na, refined with repeated washings with 100 % H_2SO_4 and distilled over Na. Bath temperature 90°/0.001 mm $d_4^{20} = 0.78584$. Table 2 gives the observed dispersional data for «Crocetane» with the values calculated for

$$R_\lambda = \frac{92.648}{\lambda^2 \div 0.7837}; \quad R_D \text{ calc.} = 94.56, \quad \text{found } 94.79$$

$$R_\lambda = \infty, \text{ calc.} = 92.389, \quad \text{found } 92.648$$

λ calc. = 885.3 ÅU; standard value for saturated hydrocarbons $\lambda_0 = 885.2$ ÅU (compare Sørensen²¹).

Table 2. Dispersion of 2,6,11,15-tetramethyl-hexadecane (Crocetane).

λ	n_λ	R_λ obs.	R_λ calc.
6678.1 ÅU	1.43791	94.297	94.305
5895.8 »	1.44055	94.791	94.785
5875.7 »	1.44063	94.806	94.800
5460.7 »	1.44243	95.142	95.149
5015.6 »	1.44513	95.646	95.627
4471.48 »	1.44930	96.422	96.428
4358.3 »	1.45038	96.623	96.635

SUMMARY

The unsaponifiable matter of the liver oil of the basking-shark was reinvestigated and cetyl-, stearyl and oleyl alcohol were demonstrated as new compounds. Pristane has been shown to be at least a nonadecane, an eicosane formula can, however, not be excluded. The synthesis of »Crocetane» was repeated and its constants found in agreement with the data of Fischer and Löwenberg. »Crocetane» in its physical constants resembles purified pristane.

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Received January 17, 1948.