

precipitates are flocculent in character, and that increase in closeness of texture and tensile strength is not always concurrent.

4—Substances like kaolin or pulverized mica, when incorporated with the pulps, do not seem to add anything to the paper produced.

5—In all the pulps, there exists a certain amount of adhesion between the fiber and the precipitated substance. This attraction is greatest for the precipitated salts of the more basic metals like calcium and magnesium, and least for the more acid metals like silver or lead. This adhesive effect may be due to some chemical action between the fiber and the precipitate.

6—The degree of porosity of all samples increased rapidly when they were heated for any length of time above 100° C., the cause of the increase of porosity being undoubtedly the contraction in bulk of the precipitated substance in the paper.

7—Tensile strength, and smoothness of surface only, seem to be enhanced by pressing with a warm iron.

8—From the identity of crysotile to "true asbestos" and the general character of the foreign substances used, the indications are pretty clear that no foreign substance either acting by itself or with others can increase the chemical stability of asbestos towards fire.

9—Under the experimental conditions set for the heat or "fire test," i. e., a temperature varying from 900–1000° C. with a time interval of 10 hours, it seems probable that no paper composed of incombustible substances containing molecular water is fire-proof or can be made fire-proof. This inference can be extended to "true asbestos" on account of its general similarity in properties and composition to crysotile.

It is true, however, that several samples of paper prepared by the writer, were not seriously impaired when exposed to a dull red heat for an hour or so. These samples still possessed considerable flexibility and could be handled, if a certain degree of care was used. There are some varieties of "true asbestos" which cling to their zeolitic(?) water with a great deal more persistence than crysotile. Paper made from asbestos of this sort would of course be quite well adapted for the preparation of, e. g., fire-proof records. In general then, when lower temperatures and shorter time limits are considered, it is very probable that an entirely satisfactory fire proof paper can be prepared.

10—In respect to fire-proof inks, solutions of ferric, chromic and cobaltous nitrates and chlorides were used. They stood the "fire test" well. According to Franz Cirkel in the Canadian Government bulletin "Asbestos—Its Exploitation and Uses" no inks up to that time prepared would stand a red heat—excepting perhaps those produced from platinum. For inks other than those prepared from platinum, one condition seems to be necessary, that the acid oxides in the paper should be kept in excess.

TULANE UNIVERSITY OF LOUISIANA, NEW ORLEANS

A HIGHLY UNSATURATED HYDROCARBON IN SHARK LIVER OIL

By MITSUMARU TSUJIMOTO

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Although usually small in their quantities, hydrocarbons seem to occur in fatty oils more frequently than hitherto considered. Some of them may possibly be utilized for the identification of individual fats and oils.

P. Matthes and O. Rohdeck¹ isolated a hydrocarbon of the composition $C_{30}H_{48}$ from cacao butter. It was considered most likely identical with amyrylene. The former chemist, together with H. Sander,² obtained a hydrocarbon from laurel oil and named it laurane. It had the composition $C_{20}H_{32}$ and formed a fine needle crystal of m. p. 69° C. from its alcoholic solution. According to another investigation of Matthes and W. Heintz,³ a hydrocarbon of the composition $C_{20}H_{32}$ (m. p. 69° C.) occurs in parsley seed oil and was named by them petrosilene. A hydrocarbon, $C_{31}H_{54}$ (m. p. 67–68° C.), is stated to occur in kôsam seed oil (Power and Lees).

In the domain of animal oils, especially in insect oils, hydrocarbons occur in considerable amounts and are characteristic of them. So, from chrysalis oil, Menozzi and Moreschi⁴ isolated two hydrocarbons: one of them had the composition $C_{28}H_{46}$, melted at 62.5° C. and was optically active, while the other melted at 41–42° C. Further, hydrocarbons were obtained from cantharide and Melolontha oils.⁵

The study of substances, beside sterols, in the unsaponifiable matter of oils and fats, so-called "sterol-free unsaponifiable matter," has lately much attracted the interest of the oil chemist. These substances consist mainly of hydrocarbons, alcohols and ketones. Thanks to the classical researches of Windaus, an effectual means for the separation of sterols from these substances was introduced into fat analysis by the use of digitonin.

The reports of J. Marcusson and G. Meyerheim,⁶ as well as P. Berg and J. Angerhausen,⁷ have satisfactorily confirmed the importance of the investigation of a sterol-free unsaponifiable matter. The latter chemists have devised, on the ground of the examination of this matter, a differentiation method of mowrah and shea butters, the distinction of which is not feasible by ordinary fat analysis.

Of marine animal oils, shark liver oil contains, as is well known, a high percentage of unsaponifiable matter, and appears to furnish a very suitable material for the above-mentioned investigation. In the literature of fats, however, we find but meagre descriptions of the oil; some statements even appear to be conflicting at a glance.

¹ Ber., 1908, 41.

² Arch. d. Pharm., 1908, 165.

³ Ber., 1909, 325.

⁴ Rend. accad. dei Lincei, 1908, 95.

⁵ Mosquito fat is likely to contain hydrocarbon (W. Normann, Chem. Rev., 1913, 187).

⁶ Z. angew. Chem., 1914, 201.

⁷ Z. Nahr. Genussm., 1914, Bd. 27, 723; Bd. 28, 73, 145.

C. Schaedler¹ and also L. E. Andes² described the sp. gr. of shark liver oils to be 0.870–0.875 (highest 0.880) at 15° C., and took oils of higher sp. gr. to be adulterated. G. Hefter,³ on the other hand; rejected the above statement as incorrect, and assigned the values 0.910–0.916 at 15° C. Both statements are correct, however, as, according to the author's experience, shark liver oils of sp. gr. 0.8644 to 0.9323 at 15° C. were met with, the value varying according to the difference of the genus and species of sharks. The great variation on the other characteristics of the oil recorded in the literature may easily be understood as well.⁴ Of the nature of the fatty acids of shark liver oil, no description is found even in the standard works on oils and fats, such as "Lewkowsch" and "Benedikt-Ulzer."

The unsaponifiable matter of shark liver oil is usually stated to consist of cholesterol. Though cholesterol is invariably found in the oil, this statement is not valid, since substances besides cholesterol, notably hydrocarbons, are found in very large quantities in some shark liver oils.

It should be mentioned that the occurrence of hydrocarbons in shark liver oil has not yet been recognized by the authorities: but in Allen's "Commercial Organic Analysis," Vol. II, Part I (1899), p. 201, the following interesting note is found: The author examined four samples of presumably adulterated shark liver oil (sp. gr. 0.8661–0.8746 at 15.5° C.) and obtained 69.9–83.5 per cent of the ether-extraction residue (unsaponifiable matter). The residue was found to be a bright yellow color like the original oil. It was free from nitrogen and nearly free from oxygen, and gave when heated an unmistakable odor of pine resin. Allen took it, apparently erroneously, to be a mixture of light rosin oil with shale or petroleum lubricating oil, which have been added to the oil as an adulterant. In my opinion, the substances must have hydrocarbons, normally present.

The first report on the occurrence of hydrocarbons in shark liver oil is, so far as I know, the one published by the author some ten years ago.⁵ The sample of shark liver oil used for examination was so-called "Kuroko-zamé" oil,⁶ obtained from the liver of a shark caught in the Sagami Sea. The oil had the sp. gr. 0.8806 at 15.5° C.; saponification value 66.85, iodine value (Wijs) 281.47, and contained 56.13 per cent of unsaponifiable matter. The unsaponifiable matter was a light yellow oil of iodine value 381, which on brominating in an ethereal solution yielded a white precipitate of bromine addition compound. By elementary analysis, the latter substance was found to consist of 26.93 per cent C, 3.94 per cent H and 69.28 per cent Br, corresponding approximately to an em-

pirical formula, C₁₀H₁₈Br₄. From this result the author inferred the existence of a highly unsaturated hydrocarbon of the composition C₁₀H₁₈ or (C₅H₉)_n.

Lately, while engaging in the examination of marine animal oils, the author has often met with shark liver oils of high content of unsaponifiable matter. Hence the previous work was again taken up and has shown that hydrocarbons are important and characteristic constituents of some shark liver oils.

In the present preliminary report, the author describes two remarkable Japanese shark liver oils ("Ai-zamé" and "Heratsuno-zamé") whose contents of unsaponifiable matter amount to about 70–90 per cent. By the present investigation, the unsaponifiable matter of the oils has been confirmed to consist chiefly of hydrocarbons. The oils are therefore not fatty oils, but hydrocarbon oil, in which a comparatively small quantity of glycerides is present.

EXPERIMENTAL

SAMPLES OF SHARK LIVER OIL USED FOR EXAMINATION

Shark liver oil (Japanese: "Samé-abura") is obtained from the liver of sharks, fish belonging to *Selachoides*. There are a great number of species of shark, but those which are caught for oil-yielding purposes are comparatively limited. The more common oil-sharks of Japan are as follows: Cat shark, Dog shark, Porbeagle, Basking shark, the Squaloid



AI-ZAMÉ (*Squalus mitsukurii*, JORDAN AND SNYDER)
Scale about 1/10

sharks (the most important shark belonging to this class is "Ai-zamé," *Squalus mitsukurii*) and the so-called "Black sharks" (*Genii Zameus*, *Lepidorhinus*, etc.).

As a result of the author's experiments, it has been observed that more or less hydrocarbons occur in shark liver oils of specific gravity lighter than 0.900 at 15° C., the content increasing as the specific gravity becomes lighter. Ai-zamé and Heratsuno-zamé oils are the lightest shark liver oils examined; hence, they contain the highest proportions of hydrocarbons.

AI-ZAMÉ OIL is obtained from the liver of "Ai-zamé,"¹ *Squalus mitsukurii* (*Squalidae*), Jordan and Snyder, one of the common squaloid sharks of Japan. The liver of this shark is very rich in oil; hence, the fish is often called simply "Abura-zamé" (Abura = oil, Zamé = shark). There are two or more varieties of *Squalus mitsukurii*. "Ai-zamé" is the name given to a variety which lives in the sea near Tokyo, notably in the Sagami and Suruga Seas. The following samples of the oil were procured:

(1) This oil was prepared in the laboratory (May 7, 1915), from the fresh liver of an "Ai-zamé," caught

¹ The meaning of "Ai" in Japanese is not exactly known. The shark is known as "Tokyo dogfish."

¹ "Technologie der Fette und Oele," 1883.

² "Animal Fats and Oils," 1904.

³ "Technologie der Fette und Oele," Bd. II (1908).

⁴ The variation may even be caused by the age and habit of fish, and also by local conditions. Cp. O. Polimanti, *Biochem. Z.*, 1915, 145.

⁵ *Kôgyô-Kwagaku Zasshi, J. Chem. Ind., Tokyo*, 1906, 953. The report was published in Japanese only.

⁶ "Kuroko-zamé" is probably a kind of "Kuro-zamé" (Black shark) of the genus *Zameus* and *Lepidorhinus*. But neither the exact genus nor species is known. (Japanese: "Samé" or "Zamé" = Shark.)

in the Suruga Sea.¹ The fish (male) was 92 cm. in length (measured from tip of snout to root of upper lobe of caudal) and weighed 8.5 kg. The color of the body of the fish was pinkish gray, becoming lighter on the underside. The snout was pointed, the spiracles large, the spines in the two dorsals rather small. The grayish white-colored liver was found to occupy the greater part of the internal cavity, the weight reaching 2120 g., *i. e.*, about 25 per cent of the total body weight. The oil was prepared by carefully heating the chopped material in a porcelain basin on a sand bath, until the moisture had been nearly expelled. The yield of the oil was about 1615 g. The oil is a pale orange-yellow liquid of a peculiar, not unpleasant odor. When spread on the skin, a fishy smell was noticed in a little while. When stirred with a drop of concentrated sulfuric acid, a fine reddish violet coloration was developed.

(2) This oil was a commercial oil, procured from a fish oil factory at Yui-chô, Suruga (Shizuoka Prefecture). It was a pale orange-yellow oil of properties very similar to Sample 1. The coloration with concentrated sulfuric acid was yellowish brown.

The following values were determined with the above two samples:

| AI-ZAMÉ OIL | (1) | (2) |
|--------------------------------|---------------|---------------|
| Sp. gr. (15° C./4° C.)..... | 0.8644 | 0.8662 |
| Solidifying pt..... | Below -20° C. | Below -20° C. |
| Acid value..... | 0.00 | 0.13 |
| Saponification value..... | 22.98 | 28.15 |
| Iodine value (Wijs)(a)..... | 344.63 | 330.35 |
| Iodine value (Hübl)(a)..... | 352.00 | 333.45 |
| Refractive index (20° C.)..... | 1.4930 | 1.4925 |
| Fatty acids..... | 10.62% | |
| Neutralization value..... | 168.52 | |
| Iodine value (Wijs)..... | 119.25 | |
| Glycerol..... | 0.52% | |
| Unsaponifiable matter..... | 90.17% | 87.32% |

(a) The time of standing was 2 and 24 hrs. with Wijs's and Hübl's solutions, respectively.

The mixed fatty acids gave, in ether solution, 16.55 per cent of polybromides, which turned black without melting at above 200° C. Glycerol was determined by the bichromate method; it was also qualitatively isolated by the acetone extraction method (Schukoff and Schestakoff). Since its percentage is a little less than the half of that calculated from the saponification value, a small quantity of some esteric compounds besides glycerides is doubtless present in the oil.

HERATSUNO-ZAMÉ OIL—"Heratsuno-zamé,"² *Deania eglantina* (Jordan and Snyder), is another squaloid shark which contains a large quantity of liver oil. The sample of the oil used for experiment was obtained from a "Heratsuno-zamé" caught also in the Suruga Sea. The fish (male) had a gray skin and a peculiar flattened head. The spine in the second dorsal was large and curved backward. It had a body length of 71.2 cm. and weighed 1.88 kg. The liver was of a grayish pink color and weighed 407 g. The oil was prepared in the same way as Ai-zamé oil. The yield was 338 g. (Nov. 29, 1915). The oil was a light yellow liquid. Its odor resembled that of Ai-zamé oil. Stirred with a drop of concentrated

¹ The date has been mentioned, because the season appears to influence the properties of fish oils.

² "Hera" = spatula, in allusion to the flat head of the fish. "Tsunô" = spine; "Zamé" = shark.

sulfuric acid, a violet-red coloration was developed. It had the following properties:

| | | | |
|------------------------------|------------|---------------------------|--------|
| Sp. gr. (15° C./4° C.).. | 0.8721 | Refractive index (20° C.) | 1.4850 |
| Solidifying pt..... | At 20° C., | Fatty acids..... | 26.59% |
| solidified to a viscous mass | | | |
| Acid value..... | 0.49 | Neutralization value.... | 168.39 |
| Saponification value.... | 52.46 | Iodine value (Wijs)..... | 73.35 |
| Iodine value (Wijs).... | 261.72 | Glycerol..... | 0.39% |
| Iodine value (Hübl).... | 259.16 | Unsaponifiable matter... | 72.88% |

The mixed fatty acids gave a very small quantity of ether-insoluble bromine additive compounds which turned black on heating. The glycerol was far less than calculated from the saponification value.

DETERMINATION OF UNSAPONIFIABLE MATTER

It is difficult and not in all cases possible to determine quantitatively the content of hydrocarbons in shark liver oils. But in the cases when no other substances besides cholesterol are present in the unsaponifiable matter together with hydrocarbons, the latter may be indirectly determined by deducting the amount of cholesterol from the total unsaponifiable matter. So far as the present shark liver oils are concerned, as will be seen later, the so-called stearyl-free unsaponifiable matter may be taken to be practically identical with hydrocarbons.

(a) DETECTION OF HYDROCARBONS—The following method, essentially a combination of the well-known Spitz-Hönig's and Bömer's methods of determina-



HERATSUNO-ZAMÉ (*Deania eglantina*, JORDAN AND SNYDER)
Scale about 1/3

tion of the unsaponifiable matter in fatty oil, has been tried with success to decide whether a given sample of shark liver oil contains hydrocarbons or not, and to determine their contents approximately. As cholesterol is very incompletely extracted by petroleum ether from soap solution, the residue of the extraction consists chiefly of hydrocarbons. When the residue has been found to be an oil, the presence of hydrocarbons may be stated with a possible certainty. The absence of hydrocarbons may be usually confirmed by a residue of a small quantity of crystals of melting point above 100° C.

The operation adopted was as follows:

5-10 g. of the sample were saponified with 50 cc. of about normal (96 per cent) alcoholic KOH in a flask with a reflux condenser on a water bath for about 30 minutes to 1 hour. On cooling, the contents of the flask were washed out into a separating funnel with 50 cc. of distilled water and shaken with 100 cc. of petroleum ether (boiling point below 65° C.) for 1 minute.

The emulsion separated quickly into two layers. After standing for about one hour, the lower layer of the soap solution was drawn off into a beaker. The petroleum ether layer was washed successively with 20 cc. of 50 per cent alcohol and distilled water, each two times, and the alcohol washings added to

the soap solution; then the petroleum ether layer drawn off into a small tared flask, the ether distilled off and the residue dried to a constant weight at 105° C. in a current of dry carbon dioxide gas. This residue consisted mainly of hydrocarbons.

The soap solution in the beaker was heated on a water bath (to expel the greater part of alcohol), then transferred into a separating funnel and extracted with each 100 cc. of ether three times. If an emulsion forms, it may be easily subsided by the addition of alcohol. The ether extracts were united and washed with distilled water; the ether was then distilled off in a tared flask, the residue dried and weighed as above.

As already stated, since cholesterol is extracted only to a small extent by petroleum ether from the soap solution, the residue of the second extraction by ether contains the main part of cholesterol. As a matter of fact, hydrocarbons are not completely extracted by a single extraction with petroleum ether, as may be expected from the distribution law. In the case of the oil of low saponification value, the petroleum ether extract gives the rough content of hydrocarbons. But if the quantity of saponifiable matter is large, considerable proportions of hydrocarbons are retained in the soap solution, so that the above number is not exact even approximately. The total unsaponifiable matter is the sum of the two extraction residues. Since a small quantity of probably hydrolyzed soap is sometimes extracted by ether together with cholesterol, the result is liable to give a little higher percentage of unsaponifiable matter with respect to the ether extract.

The following results (percentages) were obtained with the samples of shark liver oil:

| OIL | Petroleum ether extract | Ether extract | Total unsaponifiable matter |
|---------------------|-------------------------|---------------|-----------------------------|
| Ai-zamé (1)..... | 87.20 | 2.97 | 90.17 |
| Ai-zamé (2)..... | 83.06 | 4.26 | 87.32 |
| Heratsuno-zamé..... | 58.20 | 14.68 | 72.88 |

The petroleum ether extracts were faintly yellow to nearly colorless liquid, sometimes with a small deposit of apparently cholesterol crystals. The ether extracts were a pale yellow crystalline mass admixed with more or less liquid constituents (hydrocarbons). A few properties of these extracts were as follows:

| OIL | PETROLEUM ETHER EXTRACTS Iodine value (Hübl) | LIEBERMANN'S TEST | MIXED WITH ALCOHOLIC DIGITONIN SOLUTION |
|--------------------|----------------------------------------------------|---------------------------------------------|-----------------------------------------|
| Ai-zamé (1)..... | 370.80 | Bluish gray, finally dirty yellowish orange | Almost no ppt. |
| Ai-zamé (2)..... | 378.17 | | Almost no ppt. |
| Heratsuno-zamé.... | 360.82 | Bluish violet, finally dirty orange | Ppt. formed |
| ETHER EXTRACTS | | | |
| Ai-zamé (1)..... | | Reddish violet, finally dark green | Ppt. formed |
| Ai-zamé (2)..... | 187.95 | | Ppt. formed |
| Heratsuno-zamé.... | 119.80 | Violet-red, finally bluish green | Ppt. formed |

The exceptionally high iodine value of the petroleum ether extracts is very characteristic. It indicates the occurrence, in these oils, of a hydrocarbon found by the author in "Kuroko-zamé" oil for the first time (*Loc. cit.*). As may be seen below, this was actually the case with the samples of these shark liver oils.

The presence of stearyl in the ether extracts has been confirmed by the Liebermann and digitonin tests.

DETERMINATION OF CHOLESTEROL—Cholesterol was determined by the digitonin method. It has been shown by M. Klostermann and H. Opitz¹ that a part of the cholesterol in cod liver oil occurs in the form of esters; this was also found to be the case with shark liver oil.

For the determination of the total content of cholesterol, 10 g. of Ai-zamé oil, or 5 g. of Heratsuno-zamé oil were saponified with alcoholic KOH, the soap decomposed with dilute sulfuric acid and shaken with ether. The ether layer was well washed with water and then dehydrated by means of anhydrous sodium sulfate. The residue left in a flask by evaporating off the ether was mixed with 50 cc. of 1 per cent digitonin solution in 90 per cent alcohol and vigorously shaken while hot for several minutes. After standing for two or more hours, the content of the flask was filtered in a tared Gooch crucible containing layers of filter paper and cotton. As the digitonide formed an emulsion-like mixture with the hydrocarbons, about 10 cc. of chloroform were next added to dissolve the latter substances. When the precipitate had been transferred into the crucible, it was washed with 90 per cent alcohol and finally with ether. The whole was dried at 105° C. and weighed. Free cholesterol was determined by a similar method by directly treating the original oils with digitonin solution. The weight of cholesterol was calculated from the weight of the digitonide by multiplying by the factor 0.2431. The results obtained were as follows:

| PER CENT CHOLESTEROL: | Free | Combined | Total |
|-------------------------|------|----------|-------|
| Ai-zamé oil (1)..... | 0.09 | 0.46 | 0.55 |
| Heratsuno-zamé oil..... | 0.61 | 0.63 | 1.24 |

Cholesterol acetate obtained by treating the digitonide with acetic anhydride melted at 112–113° C.

If we assume the unsaponifiable matter of the shark liver oils to consist of cholesterol and hydrocarbons only, then the content of the hydrocarbons is obtained by deducting the content of cholesterol from the total unsaponifiable matter, as follows:

Ai-zamé oil (1), 89.62 per cent Heratsuno-zamé oil, 71.64 per cent

SEPARATION OF HYDROCARBONS

The petroleum ether extracts obtained in the previous experiments were presupposed to be a hydrocarbon or hydrocarbons. In order to determine whether they really consisted of a hydrocarbon or a mixture of hydrocarbons, and if any other constituents are contained in them, the following experiments were performed. Essentially, the volatility of the substances under a reduced pressure was utilized for their isolation and identification.

AI-ZAMÉ OIL (1)

FRACTIONAL DISTILLATION OF STEROL-FREE UNSAPONIFIABLE MATTER—One hundred g. of the oil were saponified with 10 g. of KOH in 10 cc. of water and 90 cc. of 96 per cent alcohol in a flask. The saponified solution, consisting of two layers (the upper hydrocarbon and the lower, chiefly soap solution), was

¹ Z. Nahr. Genussm., Bd. 27, Heft 10.

transferred into a separating funnel by washing the flask with 100 cc. of distilled water. It was then extracted with 200 cc. of light petroleum ether. The lower soap solution was drawn off, the ether layer well washed with 50 per cent alcohol and water, and then the petroleum ether distilled off.

The crude hydrocarbon thus obtained was mixed with 20 cc. of 1 per cent digitonin solution in 95 per cent alcohol and allowed to stand over night. The lower layer of hydrocarbon was then drawn off; from the upper alcoholic layer, in which the separated digitonide was chiefly suspended, the alcohol was evaporated; the residue was treated with 200 cc. of ether and mixed with the previously drawn hydrocarbon. Since digitonide and digitonin are insoluble in ether, they were deposited from the ether solution as white precipitates; these were filtered off. Finally, the ether was evaporated from the clear ethereal solution, and the stearyl-free hydrocarbon, a pale yellow oily liquid of faint peculiar odor, was left. The yield from 100 g. of the oil was 79 g. (79 per cent by weight) of a colorless liquid.

Fifty grams of the hydrocarbon thus obtained were put in a distillation flask of about 100 cc. capacity, and fractionally distilled under 8 mm. pressure.

The first drop of the distillate was observed at 280° C. bath temperature; the continuous distillation began at 240° C. (temperature of the bath, 288° C.), the thermometer soon rising to 256° C. The specific gravities and refractive indices of the original substance, as well as these fractions, were determined:

| FRACTION | 1 | 2 | 3 | 4 | 5 | TOTAL |
|----------------------------------|--------|--------|--------|--------|-----------|-------------------|
| Temperature Oil Bath..... | 291 | 289 | 289 | 288 | 286-7° C. | |
| Temperature Distillation..... | 256 | 256 | 256 | 256 | 256° C. | |
| Yield, grams..... | 8.0 | 9.7 | 9.7 | 10.4 | 11.5 | 49.3 |
| Specific gravity (15/4° C.)..... | 0.8540 | 0.8580 | 0.8589 | 0.8584 | 0.8589 | (Original) 0.8570 |
| Refractive Index (20° C.)..... | 1.4925 | 1.4963 | 1.4963 | 1.4962 | 1.4962 | 1.4955 |

Thus, the substance had a constant boiling point and its fractions showed nearly concordant values with respect to specific gravities and refractive indices. It appears, therefore, to consist mainly of a single chemical compound, although a small quantity of a substance of lower boiling point is likely to be present in the first fraction.

The elementary analysis of the substance and of Fraction 3 gave the following results:

0.1785 g. original substance gave 0.5741 CO₂, 0.1972 H₂O; C = 87.71 per cent, H = 12.36 per cent.
 0.1816 g. Fraction 3 gave 0.5817 CO₂, 0.1979 H₂O; C = 87.36 per cent, H = 12.19 per cent.
 Molecular weight of Fraction 3 (freezing point method, benzene as solvent) = 413.
 C₃₀H₅₀ requires C = 87.72 per cent, H = 12.28 per cent, mol. wt. = 410.40.
 C₃₀H₄₈ requires C = 88.17 per cent, H = 11.83 per cent, mol. wt. = 408.38.

The above results agree more closely with the formula C₃₀H₅₀.

FRACTIONAL DISTILLATION OF THE OIL—By the later experiments, it has been found that in order to obtain the hydrocarbon in pure state, the previous separation of stearyl-free unsaponifiable matter is not necessary, and the original oil or crude unsaponifiable matter may be directly distilled for this purpose, since cholesterol is nonvolatile at the temperature

and pressure at which the hydrocarbon distills over. Hence the following experiment was tried: 50 g. of Ai-zamé oil (1) were fractionally distilled under 10 mm. pressure. The distillates were colorless and contained a small quantity of free fatty acids, as may obviously be expected; the later fractions were of fairly pure quality.

| FRACTION | 1 | 2 | 3 | 4 | 5 | TOTAL |
|--------------------------------|--------|--------|--------|--------|-------------|-------|
| Temperature Oil Bath..... | 290 | 292 | 292.5 | 295-8 | 293-303° C. | |
| Temperature Distillation..... | 262-4 | 264 | 264-5 | 265 | 265° C. | |
| Yield, grams..... | 7.6 | 8.0 | 9.0 | 9.0 | 7.2 | 40.8 |
| Acid value..... | 0.99 | 0.21 | | | | |
| Refractive Index (20° C.)..... | 1.4940 | 1.4963 | 1.4965 | 1.4965 | 1.4965 | |

ELEMENTARY ANALYSIS OF THE FRACTION (4). 0.1622 g. gave 0.5183 CO₂ and 0.1765 H₂O; ∴ C = 87.15 per cent, H = 12.18 per cent.

The above composition corresponds to the formula C₃₀H₅₀.

AI-ZAMÉ OIL (2)

The stearyl-free substance was prepared by the same method as from Ai-zamé oil (1). Fifty grams of the pale yellow substance were fractionally distilled at 11 mm. pressure. The first drop was observed at 256° C. (bath 294° C.); then the thermometer rapidly rose to above 260° C. The distillates were colorless.

| FRACTION | 1 | 2 | 3 | 4 | 5 | TOTAL |
|----------------------------------|---------|--------|--------|--------|-------------|-------------------|
| Temperature Oil Bath..... | 304 | 300 | 294 | 294 | 295-306° C. | |
| Temperature Distillation..... | 265-268 | 268 | 368 | 268 | 268° C. | |
| Yield, Grams..... | 10.6 | 10.6 | 10.5 | 10.8 | 6.5 | 49.0 |
| Specific gravity (15/4° C.)..... | 0.8574 | 0.8584 | 0.8586 | 0.8587 | 0.8546 | (Original) 0.8586 |
| Refractive Index (20° C.)..... | 1.4955 | 1.4965 | 1.4965 | 1.4965 | 1.4968 | 1.4960 |

The elementary analysis gave the following results: 0.1943 g. original substance gave 0.6258 CO₂, 0.2121 H₂O; ∴ C = 87.84 per cent, H = 12.21 per cent; 0.1932 g. of fraction 3 gave 0.6230 CO₂, 0.2079 H₂O; ∴ C = 87.94 per cent, H = 12.14 per cent.

Hence, the substance consists of the hydrocarbon C₃₀H₅₀.

HERATSUNO-ZAMÉ OIL

The direct distillation method was adopted with this oil. But, since Heratsuno-zamé oil contains a far higher percentage of esteric compound than Ai-zamé oil, the separation of hydrocarbon was better accomplished by resorting to redistillation: 100 g. of the oil were distilled under 4.5 to 5 mm. pressure: the yield of the distillate was 58 g. The main part distilled at 250-260° C., but it was found that a small fraction distilling at a temperature below 250° C. at 5 mm. was also present. Then, the distillate was well washed with alkali to remove the admixed fatty acids: 40 g. of this refined substance were fractionally distilled under 5 mm. pressure. The distillates were colorless.

| FRACTION | 1 | 2 | 3 | 4 | 5 | 6 | TOTAL |
|--------------------------|--------|--------|--------|--------|--------|---------|-------|
| Temp. Oil Bath..... | 285 | 288 | 286 | 284 | 285 | 287° C. | |
| Temp. Distillation to | 248 | 248-52 | 252 | 252 | 252 | 252 | |
| Yield, grams..... | 1.4 | 6.6 | 7.2 | 7.2 | 7.2 | 7.5 | 37.1 |
| Sp. gr. (15/4° C.)..... | 0.8189 | 0.8581 | 0.8589 | 0.8589 | 0.8589 | 0.8590 | |
| Ref. Index (20° C.)..... | 1.4633 | 1.4950 | 1.4955 | 1.4962 | 1.4961 | 1.4960 | |

Fraction 1 had a lower boiling point and considerably lower specific gravity and refractive index. The

properties of the other fractions nearly coincided with those of the hydrocarbon $C_{30}H_{50}$, isolated from Ai-zamé oil.

The elementary analysis and molecular weight determination of Fractions 1 and 4 gave the following results: 0.1565 g. Fraction 1 gave 0.4925 CO_2 , 0.1928 H_2O : \therefore C = 85.82 per cent, H = 13.78 per cent; Mol. wt. = 288.

$C_{28}H_{38}$ requires C = 86.24 per cent, H = 13.76 per cent; Mol. wt. = 278.3.

Fraction 1 was thus confirmed to be a hydrocarbon, but that it really consists of a single compound requires further confirmation.

0.1880 g. Fraction 4 gave 0.6024 CO_2 , 0.2066 H_2O : \therefore C = 87.39 per cent; H = 12.31 per cent.

This fraction was thus confirmed to be the hydrocarbon $C_{30}H_{50}$.

As the result of the above-described experiments, it has been concluded that the stearyl-free unsaponifiable matter of Ai-zamé and Heratsuno-zamé oils mainly consists of a new hydrocarbon of the empirical formula $C_{30}H_{50}$. Besides this hydrocarbon, a small quantity of a hydrocarbon or hydrocarbons of lower boiling point is also present in these oils, especially in Heratsuno-zamé oil.

PROPERTIES OF THE HIGHLY UNSATURATED HYDROCARBON

So far, it has been shown that a highly unsaturated hydrocarbon, $C_{30}H_{50}$, occurs in Ai-zamé and Heratsuno-zamé oils in high proportion. Since the oils consist mainly of unsaponifiable matter, the hydrocarbon is in reality the chief constituent of these oils. A closer study of this interesting hydrocarbon will be very important from technical, and particularly from physiological points of view, as the occurrence of a large quantity of a hydrocarbon in animal oils is a matter of rarity. The experiments with this hydrocarbon, imperfect as they are, are described below:

The sample of the hydrocarbon was obtained from Ai-zamé oil (1): 100 g. of the oil were saponified and the crude hydrocarbon extracted by petroleum ether (yield, 117 g.); 108 g. of this substance were treated with 0.5 g. of digitonin, and 101 g. of the stearyl-free substance were obtained; 100 g. of the latter substance were distilled under 10 mm. pressure. Discarding this first fraction, the distillates of boiling points of 262–264° C. (temperature of the oil bath, 294–296° C.) were collected (yield, 83.5 g.); 80 g. of this distillate were redistilled under 9.5 mm. pressure. Discarding again the first fraction, the distillates of boiling points of 259–264° C. (temperature of the bath, about 300° C.) were collected (yield, 70.3 g.). This substance was used as the sample for investigation. The discrepancy of the boiling points is probably due to the rapid heating of the oil bath.

To confirm the correctness of the formula $C_{30}H_{50}$, the following elementary analysis and molecular weight determination were repeated.

(1) 0.1886 substance gave 0.6033 CO_2 , 0.2093 H_2O .

(2) 0.1553 substance gave 0.4994 CO_2 , 0.1709 H_2O .

C = 87.43 per cent, 87.70 per cent; H = 12.41 per cent, 12.30 per cent.

$C_{30}H_{50}$ requires C = 87.72 per cent, H = 12.28 per cent.

0.4020 substance in 11.7470 benzol gave $d = 0.42^\circ C$.

Mol. wt. = 408. Mol. wt. of $C_{30}H_{50} = 410.4$.

The hydrocarbon is a colorless, oily liquid which refracts light somewhat strongly. In the pure state, it is nearly odorless; but the distilled sample possesses a faint odor, apparently of decomposition products, which can be removed by washing with alkali. On keeping, it involves an odor which recalls some terpenes. Its taste is peculiar, but not so unpleasant. It is readily soluble in ether, petroleum ether, carbon tetrachloride and acetone; in cold alcohol and glacial acetic acid, it is sparingly soluble. When heated over a flame, the hydrocarbon burns with a smoky, luminous flame, emitting a resinous smell.

BOILING POINT—Under ordinary pressure, the hydrocarbon cannot be distilled without decomposition. Boiling point (10 mm. pressure), 262–264° C.; (5 mm. pressure) 252–254° C.

SOLIDIFYING POINT—Kept at $-20^\circ C$. for one hour, it remained clear; at $-75^\circ C$., it solidified to a white wax-like mass.

SPECIFIC GRAVITY—At $15^\circ/4^\circ C$., 0.8587.

IODINE VALUE—388.12 (Wijs), 398.70 (Hübl).

Theory requires for $C_{30}H_{50}I_{12}$, iodine value = 371.10.

REFRACTIVE INDEX—($20^\circ C$.) 1.4965.

OPTICAL ROTATION—inactive.

HEAT OF COMBUSTION—(Hempel's calorimeter), 10,773 cal. per g.

COLOR REACTION—By Liebermann's test (solution in acetic anhydride and addition of a drop of concentrated sulfuric acid), the fresh hydrocarbon gave only a faint pink coloration in the lower (acetic anhydride) layer.

DRYING PROPERTY—The hydrocarbon dries like the vegetable drying oils. The pure hydrocarbon requires a long time to dry at the ordinary temperatures. That which had been treated with 1 per cent of cobalt resinate, dried in about 10 days (in winter) to a colorless, smooth film which possessed a firmness superior to those of the fatty oils. At $100^\circ C$., a thin layer of the hydrocarbon dried in about 2 hours.

ACTION OF ACIDS—Concentrated sulfuric acid attacked the hydrocarbon violently with evolution of heat, changing it to a dark reddish brown mass. Concentrated nitric acid acted not so violently. A very violent reaction was observed by a mixture of concentrated nitric and sulfuric acids.

ACTION OF ALKALIES—Even caustic alkalies had practically no action at $100^\circ C$.

ACTION OF SULFUR CHLORIDE—A yellow solid mass was obtained.

PICRATE COMPOUND—No definite compound was obtained.

BROMIDE ADDITIVE COMPOUND—0.6326 g. of the hydrocarbon was dissolved in 10 cc. of ether in a flask, the solution cooled with ice, and 0.55 cc. of bromine was added drop by drop. After standing for 2 hours,

the precipitate was filtered on a tared filter paper, washed with about 70 cc. of ether and dried *in vacuo*, over sulfuric acid, until the weight became constant.

The yield of the insoluble bromide was 0.5630 g., *i. e.*, 89.00 per cent of the sample.

0.1467 substance gave 0.1413 CO₂, 0.0496 H₂O.

0.1587 substance gave 0.2600 AgBr (Carius method).

C = 26.27 per cent, H = 3.78 per cent, Br = 69.75 per cent.

C₃₀H₅₀Br₁₂ requires C = 26.78 per cent, H = 3.72 per cent, Br = 69.50 per cent.

The filtrate from the above precipitate was treated with sodium thiosulfate solution, in order to remove the excess of bromine, washed with water and dried with anhydrous sodium sulfate. On evaporating off the ether, a viscous solid was left, which became a white powder on grinding. The yield was 1.6070 g., *i. e.*, 254.00 per cent of the original sample. The soluble bromide was then treated with a few cc. of ether to remove admixed impurities, and analyzed.

0.1157 substance gave 0.1117 CO₂, 0.0391 H₂O.

0.1664 substance gave 0.2710 AgBr.

C = 26.33 per cent, H = 3.78 per cent, Br = 69.30 per cent.

This substance is, therefore, identical with the insoluble portion.

The total yield of the bromide is thus 333 per cent. Theory requires 327.60 per cent, nearly a good accord. The bromine additive compound is a white powder, turning black at about 170° C. and decomposing at 176–177° C. It is sparingly soluble in organic solvents, especially when dried.

The bromide obtained from "Kuroko-zamé" oil described in the introductory note, is the same substance as this brominated hydrocarbon. The analytical results given there correspond more closely with C₃₀H₅₀Br₁₂ than C₁₀H₁₈Br₄. Therefore, the hydrocarbon in the above-named shark liver oil must have been the hydrocarbon C₃₀H₅₀.

HYDROGENATION COMPOUND—3.3115 g. of the hydrocarbon were dissolved in 30 cc. of ether in a shaking bottle. To this solution, 0.5 g. of Loew's platinum black was added. The bottle was then connected to a gas burette, which in turn was connected with a hydrogen holder. Hydrogen was prepared from pure zinc (Merck) and dilute sulfuric acid, and before entering into the burette, it was washed and dried by bottles containing a solution of potassium permanganate and concentrated sulfuric acid. By vigorously shaking the bottle, hydrogen was conducted into it under the mercury pressure. After 2 hrs. 40 mins., the absorption ended; the volume of hydrogen absorbed, together with a little leakage, was 1120 cc. The hydrogenated compound, left on evaporating off ether, was found to be a colorless oil, resembling in its appearance the so-called liquid paraffin. It had the following properties: Sp. gr. at 15/4° C., 0.8125; b. p. (10 mm. pressure), 274° C.; solidifying pt.: at -20° C. it remained clear and mobile; at -80° C., solidified to a transparent jelly which at -35° C. regained its mobility; ref. index at 20° C., 1.4525. It was not readily acted on by concentrated sulfuric acid, even at 100° C.

ELEMENTARY ANALYSIS: (1) 0.1610 substance gave 0.5022 CO₂, 0.2114 H₂O.

(2) 0.1910 substance gave 0.5959 CO₂, 0.2523 H₂O.

C = 85.07, 85.09 per cent; H = 14.69 per cent, 14.78 per cent.

C₃₀H₆₂ requires C = 85.21 per cent, H = 14.79 per cent.

Molecular weight determination, by freezing point method, 0.2982 substance in 11.1030 benzol, d = 0.31° C. Mol. wt. = 433.

C₃₀H₆₂ requires mol. wt. = 422.5.

The substance has therefore been confirmed to be a compound of the empirical formula C₃₀H₆₂. As C₃₀H₆₂ is of a type of the general formula C_nH_{2n+2}, it appears that the hydrocarbon C₃₀H₅₀ belongs to the aliphatic compounds.

PREPARATION AND APPLICATION OF THE HYDROCARBON

Ai-zamé and Heratsuno-zamé oils are produced in somewhat large quantities in certain districts of Japan, especially in Shizuoka Prefecture. So the preparation of the hydrocarbon is practicable even for commercial purposes; all the more so, as the author has lately ascertained the presence of the hydrocarbon in other shark liver oils besides the above mentioned ones. (See below.)

The preparation of the hydrocarbon is, according to the author's experience, best conducted by distilling the oil under reduced pressure, or by means of superheated steam.¹ Since cholesterol is comparatively nonvolatile, the distilled hydrocarbon contains more or less admixed fatty acids. Therefore, by subsequent washing with alkali, it can be obtained in nearly pure state.

As to the use of the hydrocarbon, only a few experiments have been tried as yet, and exact descriptions cannot be given here. It may be mentioned, however, that for technical purposes, the hydrocarbon may be used for paints, varnishes, lithographic inks, and oil colors. The hydrogenated product, which in its appearance is very similar to so-called liquid paraffins and, at the same time, far more stable for cold, will be a useful material for lubrication of machines.

The medicinal use of the hydrocarbon, possible for the same purpose as cod liver oil, will perhaps be most interesting, but careful researches are necessary to settle this question.

CONCLUSIONS

The results of the present investigation have confirmed that two Japanese shark liver oils, Ai-zamé and Heratsuno-zamé oils, contain very high proportions of unsaponifiable matter which mainly consist of a new highly unsaturated hydrocarbon of the formula C₃₀H₅₀. It will be rather premature to make any further statement, but certainly a remarkable quantity of a hydrocarbon which in reality is the essential constituent of the oil itself, occurs in oils of such vital organs as the liver of the fish. It may be added here from the author's latest experiments that the hydrocarbon also occurs in the liver oils of so-called "Black sharks," basking sharks and another

¹ Japanese Patent, No. 28,143, Aug. 4, 1915.

shark (probably *Triakis scyllium*—Japanese; “Koro-zamé”). Sharks belonging to Squalidae appear to contain the hydrocarbon more frequently. But, the data obtained hitherto are yet too scanty to allow a generalization as to the genus and species of sharks.¹

To solve the question, why such a hydrocarbon is present in the liver of some fish, is probably very difficult, and presents an important problem for physiological chemistry. It is interesting to note that the bromine content of the bromine additive compound of the hydrocarbon is nearly identical with those of the polybromides of the highly unsaturated fatty acids² in marine animal oils, *viz.*, about 70 per cent.

In the case of the present shark liver oils, the place of the highly unsaturated fatty acids is taken, as it were, by the hydrocarbon of a nearly equally high unsaturation. The liver is a storage organ for fat and consequently a source of energy of the fish. If a speculation may be allowed, the hydrocarbon, which evolves far more heat of oxidation than the glycerides, must have been formed in the liver of the fish from the necessity of local conditions. Little as the habit of the sharks is known, it is pretty certain that they are comparatively deep sea dwellers.

For technologists, the hydrocarbon is not less important. The “drying” properties of glycerides (vegetable drying oils) and certain phenols, such as Urushiol, C₂₀H₃₀O₂, the chief constituent of Japanese lacquer, are well known, but yet those of the hydrocarbons have not been investigated.

In a second paper on this subject, the author hopes to publish the results of the latter experiments, performed on other shark liver oils, and an examination of the further properties of the hydrocarbon.

ADDENDUM—After the foregoing report had been written, the author read a paper by H. Mastbaum (Lisbon), entitled “Hydrocarbon in Fish Liver Oils,” through *Chemical Abstracts*, 10, No. 2, just arrived. The author has examined two liver oils from *Centrophorus granulosus* and *Scymnus lichia*, and found 80–90 per cent of oil with the characteristic of mineral oil. The unsaponifiable portion of the oil from *Scymnus lichia* is said to have boiled at 205–330° C. (under the ordinary pressure²). As no analytical result of the unsaponifiable matter is given, it is not possible to decide whether they contained the hydrocarbon C₃₀H₅₀ or not. But, as the former shark belongs to *Squalidae*, the hydrocarbon in the oil is doubtless the same. From this report, it may be seen that the liver oil of foreign squaloid shark also contains hydrocarbon. Although not the only one of

the kind, the family *Squalidae* seems to have a more important relation to the hydrocarbon than other families. From such reasons, and particularly from the fact that the author has discovered the hydrocarbon first in the liver oils of the squaloid sharks, he proposes the name “*Squalene*” for the hydrocarbon.

IMPERIAL INDUSTRIAL LABORATORY
YETCHIU-SHIMA, TOKYO, JAPAN
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AERATION METHOD FOR AMMONIA

By B. S. DAVISSON, E. R. ALLEN AND B. M. STUBBLEFIELD

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Investigations in soil biology require frequent exact determinations of ammonia in the presence of organic nitrogenous compounds. The problem is therefore the same as that encountered in general biological chemistry with the additional difficulty that large amounts of solutions must be used in soil biological work because of the smaller percentage of ammonia in the material under examination. For instance, in biological chemistry, in the determination of ammonia in urine, 25 to 50 cc. portions of urine are taken, whereas in soil biology 250 cc. of soil extract or of physiological solution are frequently necessary to give a measurable amount of ammonia nitrogen.

The matter of the separation of ammonia from organic nitrogen has been the subject of a very large amount of study by biological chemists. The tendency of all recent work has been toward the use of an air current to transfer the ammonia from cold alkaline solution into acid receivers and determination of the ammonia by titration (or Nesslerization) of the receiver contents. The use of larger amounts of solutions naturally renders the removal by the air current slower. Moreover the matter of the kind and concentration of alkali is still a matter of controversy among workers in this field. Obviously, the lowest hydroxyl ion concentration compatible with complete removal of ammonia under workable conditions is desirable since decomposition of the organic nitrogenous material is roughly proportional to the concentration of hydroxyl as ion.

The use of different alkalis, and their effect on the rapid removal of ammonia from large amounts (250 cc.) of solution by means of a vigorous air current, and its subsequent quantitative collection and measurement constitutes the experimental work reported in this paper. It was desired to obtain a method by which the ammonia can be recovered in a period of 2½ to 3 hrs. and, if possible, the use of an alkali imparting a low concentration of hydroxyl as ion to the solution.

HISTORICAL

Folin¹ outlined a method for determining ammonia in urine: 25 cc. of urine were aerated for 1 to 1½ hrs. over 1 g. of sodium carbonate and 8 to 10 g. of sodium chloride. The rate of air used was 600 to 700 liters per hour. The ammonia was absorbed in standard acid and titrated with standard alkali. He devised the well-known Folin absorption tube by which he was able to get complete absorption of the ammonia. He used MgO for some determinations

¹ Oils prepared from the following sharks have been found not to contain the hydrocarbon. Cat shark (*Heterodontus japonicus*), Blue shark (*Isuroopsis glauca*), Porbeagle (*Lamna cornubica*), Saw shark (*Pristiophorus japonicus*), Hammer-headed shark (*Sphyrna zygaena*), and Angel-fish (*Squatina japonica*). Nor has the hydrocarbon been found in the liver oils from the following rays: Ray (*Raja kencajei*), Sting-ray (*Dasyatis akajei*), Eagle-ray (*Myliobatis tobiei*), and *Discobatus sinensis* (Japanese; “Uchiwa-zamé”).

Shark liver oils from probably *Squalus sucklii* contained 12.00 to 21.64 per cent of unsaponifiable matter, not volatile at 300° C. under 10 mm. pressure. The nature of the substance has not yet been ascertained. Details will be published in a next report.

² Acids of the series C_nH_{2n}—8O₂ and C_nH_{2n}—10O₂.

¹ Folin, *Z. physiol. Chem.*, 37 (1912), 161.