CXIV.—*The Unsaponifiable Matter from the Oils of Elasmobranch Fish. Part V. The Constitution of Squalene as deduced from its Degradation Products.*

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SINCE our previous publication on this subject (Heilbron, Kamm, and Owens, J., 1926, 1630), in which reference was made to a preliminary communication by André and Canal (*Compt. rend.*, 1925, **181**, 612), a detailed account of their work has appeared (*Ann. Chim.*, 1927, **7**, 69). These authors seemingly hold the view that either squalene is not a single substance or, if it is, its formula is best represented as $C_{28}H_{46}$ (or possibly $C_{27}H_{44}$) but not as that of a dihydrotriterpene, $C_{30}H_{50}$. Criticism of our results is based on the ground that analyses of the hydrohalides are insufficient to allow of a decision being made with regard to the constitution of the hydrocarbon owing to the salts being incompletely saturated. If this be so, it is certainly a striking coincidence not only that the majority of André and Canal's analytical values for both the hydrochlorides and the hydrobromides indicate the $C_{30}H_{50}$ formulation, but that in more than fifty analyses of the isomeric hydrochlorides, prepared in these laboratories from squalene obtained from various sources, the percentages of carbon, hydrogen, and halogen are in every case in agreement with this formula (compare also Tsujimoto, *J. Ind. Eng. Chem.*, 1916, **8**, 889). The results obtained by André and Canal, for details of which their original paper must be consulted, are so conflicting that criticism is rendered difficult. That they themselves feel some uneasiness regarding their own inferences is apparent from a further communication (*Bull. Soc. chim.*, 1928, **44**, 371) in which they seemingly come to our conclusion (Heilbron, Kamm, and Owens, *loc. cit.*) that the two hydrochlorides of different melting point are actually isomerides and are not salts of neighbouring homologues. As regards the formula $C_{28}H_{46}$, this is now left an open question.

We would point out that the dihydrotriterpene structure for squalene was not advanced *solely* on the analytical values of the hydrohalide salts : other factors were taken into consideration. Of these, its typical terpene character as evidenced by (*a*) the pyrogenic decomposition products, (*b*) the ease with which it undergoes ring closure, and (*c*) the substances obtained by the action of ozone (Majima and Kubota, *Jap. J. Chem.*, 1922, **1**, 19), may be cited.

The present communication deals with work primarily undertaken with the idea of proving that under all methods of attack squalene yields decomposition products typical of those to be anticipated from a triterpene hydrocarbon. The results actually obtained have, however, enabled us to ascribe definite structural formulæ to the isomeric hydrocarbons of which it is composed.

In the first place we have repeated our previous work on the distillation of squalene, using larger quantities of the hydrocarbon and examining especially the low-boiling fraction from which Chapman (J., 1918, **113**, 458; 1923, **123**, 769) obtained a cyclic hydrocarbon similar in physical characteristics to *cyclo*dihydromyrcene (compare also Majima and Kubota, *loc. cit.*). Fractions agreeing closely with those obtained by these authors have been isolated, but we consider them to be constant-boiling mixtures rather than individual substances.

We have also repeated the hot-wire method of decomposition employed by Chapman (J., 1923, **123**, 769) and here again our experiments have failed to confirm the existence of a single substance such as the diolefinic hydrocarbon, heparene ($C_{10}H_{18}$), isolated by him. Indications of the presence of an unsaturated open-chain terpene (myrcene ?) have been furnished by an experiment in which the monoterpene fraction was boiled with formic acid, whereby a high-boiling hydrocarbon closely resembling dimyrcene in physical characteristics was separated (Semmler and Jones, *Ber.*, 1913, **46**, 1566). Its appearance in this manner is obviously the result of the polymerisation of some constituent of lower molecular weight in the original mixture.

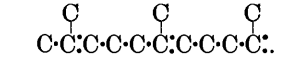
In the hope of being able definitely to identify some particular monoterpene, we made attempts with each fraction of seemingly homogeneous nature to isolate a solid derivative, such as the bromide or the hydrochloride, but only uninviting oily products were obtained.

Action of Ozone upon Squalene.—By the action of ozone upon squalene, Majima and Kubota (*loc. cit.*) readily obtained a hexaozonide from which carbon dioxide, acetone, acetone peroxide, formaldehyde, formic acid, succinic acid and lævulic acid were produced on decomposition with water. In addition, two acidic substances were isolated in small quantity, to which the formulæ $C_6H_{10}O_5$ and $C_8H_{14}O_6$ were ascribed, without, however, their structures being determined. We have repeated this investigation and have not only confirmed the formation of all the above products, but ascertained that the two supposed acids are in reality peroxides. The compound of lower molecular weight, m. p. 195°, has actually the formula $C_5H_8O_4$ and is identical with lævulic aldehyde peroxide

(compare Harries, *Ber.*, 1905, **38**, 1201): the substance $C_8H_{14}O_6$, m. p. 136°, appears to be a complex peroxide of methylheptenone.

Oxidation of Squalene with Potassium Permanganate.—The action of potassium permanganate upon squalene was examined by Chapman (J., 1923, **123**, 769), who, however, was unable to isolate any derivatives likely to throw light on its constitution. On treatment with alkaline permanganate the formation of "a little acetone together with a trace of some fragrant ketone" was noted, but the bulk of the hydrocarbon either remained unattacked or was completely destroyed according to the conditions employed.

The results obtained by us substantially confirm these findings, but, by treatment of squalene in acetone solution with solid potassium permanganate at room temperature, two ketones have been isolated in minute amount, one of which we have definitely characterised as dihydro-ψ-ionone (geranylacetone). The formation of this ketone adds an important link to the evidence of the terpenoid nature of squalene, proving as it does the presence of the carbon skeleton



The other ketone is almost certainly methylheptenone, and its presence accords well with the isolation of the $C_8H_{14}O_6$ peroxide. An acid portion has also been obtained from which succinic acid has been separated.

The greater part of the squalene was recovered unchanged and was further treated with an excess of potassium permanganate in the heat. Even under these drastic conditions about 40% of the squalene was unacted upon, the remainder being completely burnt to carbon dioxide. The residual squalene yielded a hydrochloride (m. p. 105—116°) from which, after *one* crystallisation, mainly the high-melting isomeride was obtained. The ready formation of this hydrochloride, which is only slowly accumulated from untreated squalene, adds further support to our contention (Heilbron, Kamm, and Owens, *loc. cit.*) that squalene is a mixture of two isomerides and suggests that one isomeride is more readily broken down than the other.

Oxidation of Squalene with Chromyl Chloride.—The additive products obtained by treating a solution of squalene in carbon disulphide with chromyl chloride in the same solvent furnished, on decomposition with water, small amounts of formaldehyde, acetaldehyde and succinic acid, together with much unworkable resinous material. In the last respect the results are similar to those recorded by Chapman (*Analyst*, 1927, **52**, 622) but, contrary to the findings of this author, the completely saturated addition product was obtained with twelve molecules of chromyl chloride instead of eight.

Squalene Dodecabromide.—As early as 1916, Tsujimoto (*J. Ind.*

Eng. Chem., **8**, 894) found that squalene formed an addition compound with bromine, m. p. 170—176° (decomp.), to which he ascribed the formula $C_{30}H_{50}Br_{12}$. Chapman (J., 1917, **111**, 63) also has described a dodecabromide of spinacene, m. p. 185°, which he found was divisible into two portions differing in solubility but having the same composition ($C_{30}H_{50}Br_{12}$). In a further paper (J., 1918, **113**, 458) this author commented on the fact that the purified dodecabromide was the sole exception to his amended formula for spinacene ($C_{29}H_{48}$), but ultimately, by crystallisation of the less soluble portion from a mixture of tetrachloroethane and alcohol, a compound conforming to this formula was obtained (J., 1923, **123**, 770).

In the present work, squalene from several sources was carefully brominated at — 25°, and the colourless bromide separated into a high- and a low-melting product by means of ethyl acetate. The latter, which was the more soluble, rapidly darkened in moist air and, owing to decomposition, could not be recrystallised. On the other hand, the high-melting, insoluble dodecabromide (m. p. 198°) was stable and gave analytical values definitely fixing its formula as $C_{30}H_{50}Br_{12}$.

Discussion of Results.

Although we have failed to obtain any direct information, leading to the elucidation of the structure of squalene, from the results of our examination of the low-boiling pyrogenic decomposition products, there can be little doubt that these constitute a complex mixture of mono- and dihydromono-terpenes. That a mixture of this nature should exist is in strict accord with our view that squalene is a dihydrotriterpene, and finds confirmation both in the work previously carried out (Part I) and in our isolation of methylheptenone and dihydro-ψ-ionone. The formation of these ketones together with the appearance of acetone, lævulic aldehyde and lævulic acid among the decomposition products of the ozonide affords striking confirmation of our previously suggested structure (Heilbron, Kamm, and Owens, *loc. cit.*) for one form of squalene (I).

(I.) $CMe_2{:}CH{\cdot}CH_2{\cdot}[CH_2{\cdot}CMe{:}CH{\cdot}CH_2]_4{\cdot}CH_2{\cdot}CMe{:}CHMe$

(II.) $CMe_2{:}CH{\cdot}CH_2{\cdot}[CH_2{\cdot}C(:CH_2){\cdot}CH_2{\cdot}CH_2]_4{\cdot}CH_2{\cdot}CMe{:}CHMe$

Such a structure, however, fails to account for the formation of carbon dioxide (8·5%), formaldehyde, formic acid and succinic acid (28%) in the same decomposition. We are therefore forced to the conclusion that at least one isomeride must exist, and for this we suggest formula (II), a structure which has already been deduced from the hydrogenation of squalene (Heilbron, Hilditch, and Kamm, J., 1926, 3131).

The formation of formaldehyde and acetaldehyde in the chromyl chloride reaction is also in harmony with the above formulations, for, whereas the former obviously arises from the $>C{:}CH_2$ group (formula II), the latter is produced by scission at the double bond of the terminal group $CMe{:}CHMe$ common to both structures.

The weight of the oxidation products isolated by us from the ozone treatment accounts for fully 90% of the squalene used (Majima and Kubota obtained a somewhat higher percentage)—a fact which, taken in conjunction with the other evidence now provided, appears to us to refute André and Canal's contention that squalene has a molecular formula other than $C_{30}H_{50}$.

Experimental.

Squalene from the liver oil of *Centrophorus granulosus* was originally employed, but later the hydrocarbon was extracted from *Spinax niger* and *Scymnorhinus lichia*. In no case, however, was any difference observed in the characteristics of the pure hydrocarbon, which was worked up by the distillation process previously described (Heilbron, Kamm, and Owens, *loc. cit.*).

Decomposition of Squalene by Heat.—The hydrocarbon was distilled in portions not exceeding 400 g. at 300—350 mm. in an atmosphere of nitrogen. Excluding the hemiterpene fraction, which has previously been shown to consist mainly of the amylene $CMe_2{:}CHMe$, a typical decomposition yielded a clear, golden-yellow, mobile liquid (yield, 70%). The residue consisted of a dark brown, very viscous liquid which hardened to a horny mass on standing. The distillate was roughly fractionated at 20 mm. in an atmosphere of nitrogen and the monoterpene portion ($n_D^{18\cdot5°}$ 1·4634) boiling between 40° and 100° (30% of the total) was collected and subjected to careful fractional distillation. No evidence of the presence of any homogeneous fraction was obtained, the temperature rising gradually throughout the whole operation. The most abundant fraction, b. p. 60—63°/15 mm. (Majima and Kubota find 64—66°/18 mm. for their hydrocarbon similar to *cyclo*dihydromyrcene), was obtained as a pale yellow liquid (30%), $d_4^{17\cdot5°}$ 0·8225, $n_D^{20°}$ 1·4619 (Found: C, 87·5; H, 12·4; iodine value, 316. $C_{10}H_{16}|_2^=$ requires C, 88·2; H, 11·8%; iodine value, 373. $C_{10}H_{18}|_1^=$ requires C, 87·0; H, 13·0%; iodine value, 184). The non-homogeneous character of the fraction indicated by the analytical data was confirmed by refractionation from a Willstätter flask; the seemingly constant-boiling liquid then distilled over a much wider range (162—177°/740 mm.) and the physical constants of the collected fractions changed uniformly with rise in boiling point.

Decolorisation of the Monoterpene Fraction.—As all the distillates

obtained by the above method of decomposition were of a bright yellow colour, appropriate fractions from different experiments were mixed together and thrice distilled over sodium, whereby colourless, pleasant-smelling, mobile liquids resulted. Of the larger fractions, the following are of especial interest, as they closely approximate in physical characteristics to those recorded both by Chapman and by Majima and Kubota (*locc. cit.*).

(1) B. p. 167—170°/759 mm., $d_{16^\circ}^{16^\circ}$ 0·8188, $n_D^{20^\circ}$ 1·4589 (Found: C, 87·4; H, 12·4%; bromine value, 138; iodine value, 270. $C_{10}H_{18}I_1^{=}$ requires bromine value, 116; iodine value, 184).

(2) B. p. 174—176°/759 mm., $d_{16^\circ}^{16^\circ}$ 0·8262, $n_D^{20^\circ}$ 1·4624 (Found: C, 87·3; H, 12·7%; bromine value, 148; iodine value, 263).

The bromine values are in fair agreement with the figures given by Chapman (J., 1918, **113**, 464) for the mono-olefinic cyclic hydrocarbon obtained by the decomposition of squalene (spinacene) in the presence of sodium. On the other hand, the iodine absorption values found by us correspond to the addition of three atoms of iodine per molecule of hydrocarbon. It is thus clear that evidence based on either bromine or iodine absorption cannot be relied upon as a sure criterion of structure. Attention must also be drawn to the results of ultimate analysis. Whereas both the above-mentioned authors give analytical figures in agreement with $C_{10}H_{18}$, our values for carbon (over 15 analyses on different fractions) invariably correspond to the requirements of a hydrocarbon mixture of $C_{10}H_{16}$ and $C_{10}H_{18}$ molecules, the relative proportion of which remains almost constant no matter how far the degree of fractionation is carried.

Hot-wire Decomposition of Squalene.—This was carried out according to Chapman's directions (J., 1923, **123**, 769) in a specially constructed Jena flask containing in the neck a mesh of fine platinum wire which was maintained at a dull red heat during the distillation process by means of an electric current. Squalene in three batches of 140 g. was distilled under a pressure of 45—50 mm. The bright yellow, mobile distillate (273 g.) was divided into two portions and worked up (*a*) by direct fractional distillation and (*b*) by steam distillation.

(a) *Fractional distillation.* The crude distillate was separated into mono-, sesqui- and higher terpene fractions. The monoterpene fraction was examined in great detail but gave the same indefinite fractions as were obtained by the direct pyrogenic decomposition.

(b) *Steam-volatile portion* (42 g. from 133 g. of crude material). Contrary to Chapman's finding (*loc. cit.*), here again we failed to isolate any definite major portion on fractionation, the boiling point rising continuously throughout the distillation (110—171° at the

ordinary pressure). A fraction (10 g.), b. p. 165—171°/755 mm., was ultimately collected as a bright yellow liquid, d_{15}^{15} 0·8092, n_D^{20} 1·4610 (Found: C, 87·4; H, 12·4%; iodine value, 345, 355). The physical constants agree with those given for heparene ($C_{10}H_{18}$), but the yellow colour is completely removed on distillation over sodium and cannot be attributed to a definite structural arrangement of the fraction as a whole.

Action of Formic Acid.—A fraction boiling between 60° and 100°/20 mm. (20 g.) was heated for 24 hours with 99% formic acid (25 g.) on a steam-bath. The deep purple solution was diluted with water, rendered alkaline with sodium carbonate, and extracted with ether. After removal of the solvent from the dried extract, the brown oil was fractionally distilled as follows: (*a*) 160—170°/759 mm. (2·5 g.); (*b*) 171—176°/759 mm. (4 g.), d_{20}^{20} 0·8337, n_D^{20} 1·4582; (*c*) 60—85°/3 mm. (0·5 g.); (*d*) 125—130°/3 mm. (4 g.), d_{20}^{20} 0·9098, n_D^{20} 1·4998. The last fraction was obtained as a viscous yellow liquid having the typical characteristics of a diterpene (Found for fraction *d*: C, 87·8; H, 12·0. $C_{20}H_{32}$ requires C, 88·2; H, 11·8%).

Preparation of Squalene Hexaozonide.—A stream of oxygen containing approximately 5% by volume of ozone was passed at the rate of 6—8 litres per hour through a solution of squalene (30 g.) in dry chloroform (120 g.) maintained at 0°, until excess of ozone was detected at the outlet of the apparatus (72 hours). The solvent was carefully removed below 35° under reduced pressure so as to avoid risk of explosion due to the unstable nature of the hexaozonide. The deposition of crystals of succinic acid from the chloroform solution of the ozonide, as described by Majima and Kubota (*loc. cit.*), was only observed on two occasions. The acid was identified by direct comparison with an authentic specimen of succinic acid, the mixture melting at 179—181°. The ozonide was taken up in dry ether and reprecipitated with light petroleum. The supernatant liquid was decanted and the remaining traces of volatile solvent were removed under reduced pressure. The ozonide formed a colourless viscous syrup (yield, 48 g.).

Decomposition of Squalene Hexaozonide.—This was carried out exactly as described by Majima and Kubota, but it was found difficult to isolate the acetone peroxide in the quantity mentioned by these authors. It was further observed that, if the ozonisation was carried out at room temperature, a larger proportion of lævulic acid was formed at the expense of the two supposed acids, thus pointing to some definite relationship between them. Acetone and carbon dioxide were readily identified, the former being isolated as its semicarbazone (2·5 g.), m. p. 186—187°, and the latter estimated as barium carbonate (equivalent to 2·5 g. of carbon dioxide).

Among the products volatile in steam, acetone peroxide, m. p. 132—134°, was isolated as a crystalline deposit (0·5 g.), and the presence of lævulic aldehyde was detected by the preparation of its phenylmethyldihydropyridazine, m. p. 194—196°. Formic acid was also readily identified. The residual solution obtained after steam distillation was worked up exactly as described by Majima and Kubota and succinic acid (8·4 g.), lævulic acid (11·0 g.), and an alkali-soluble, waxy solid consisting of a mixture of the two unknown "acids" of these authors (3·5 g.) were isolated. These "acids" were separated by boiling benzene, in which the higher-melting compound was almost insoluble, and each was purified by repeated crystallisation from water. Lævulic aldehyde peroxide was obtained in colourless needles, m. p. 195° (literature values, 190° to 196°), readily soluble in boiling water, insoluble in benzene [Found: (micro.) C, 45·6; H, 6·3. Calc. for $C_5H_8O_4$: C, 45·4; H, 6·1%]. The substance liberates iodine from dilute potassium iodide solution in the cold and also reduces Fehling's solution. When the peroxide is boiled for 12 hours with water, a clear solution is formed; on treatment with a dilute acetic acid solution of *p*-nitrophenylhydrazine, the *p*-nitrophenylhydrazone of lævulic acid is deposited, m. p. 174—175°.

Methylheptenone peroxide (?) crystallises from boiling water in colourless plates, m. p. 136° (Majima and Kubota give 132—134°) (Found: C, 46·5, 46·5; H, 7·3, 6·9. $C_8H_{14}O_6$ requires C, 46·6; H, 6·8%).

On being heated with water in a closed tube for 8 hours at 140°, the peroxide decomposes, giving a colourless solution devoid of aldehydic reactions and acid to methyl-orange. Lævulic acid *p*-nitrophenylhydrazone, m. p. 169—170°, was isolated from the solution in the usual manner and identified by the melting point, 171—173°, of a mixture with an authentic specimen (m. p. 173—174°).

Oxidation of Squalene with Potassium Permanganate.—A solution of squalene (200 g.) in carefully purified acetone (400 g.) was treated at room temperature with continuous stirring with finely powdered potassium permanganate (300 g.), added in small quantities during 18 hours. The mixture was diluted with water, sulphur dioxide passed into the solution to remove manganese dioxide, and the whole repeatedly extracted with ether. The ethereal solution was separated into acid and neutral portions by extraction with dilute sodium carbonate solution.

Succinic acid. After concentration the alkaline solution was made acid. The precipitated solid crystallised from ethyl acetate in colourless crystals, m. p. 185°, which were identified as succinic acid (melting point of a mixture).

Neutral portion. The ethereal extract, after drying and removal of solvent, was fractionally distilled and yielded a small fraction, b. p. 90—95°/18 mm. The greater portion of the distillate consisted of unchanged squalene, which was again treated with an excess of potassium permanganate in *boiling* acetone solution in presence of magnesium sulphate until all action had ceased. Despite the drastic conditions employed, about 40% of the squalene was recovered, the remainder having been completely burnt to carbon dioxide. The recovered squalene ($n_D^{20°}$ 1·4980) yielded a crude hydrochloride, m. p. 105—116°, which after one recrystallisation from hot ethyl acetate, gave mainly the high-melting squalene hydrochloride (m. p. 121—125°).

Dihydro-ψ-ionone. The low-boiling product had a pleasant pear-like odour (compare Chapman, J., 1923, **123**, 771) and reacted with semicarbazide acetate. The crude semicarbazone (2 g.) was taken up in light petroleum and separated from a small insoluble residue. The filtrate slowly deposited a colourless solid, which was recrystallised from aqueous methyl alcohol. The pure semicarbazone, m. p. 95°, was identified as dihydro-ψ-ionone semicarbazone by comparison with a synthetic specimen prepared from geraniol according to Forster and Cardwell's method (J., 1913, **103**, 1341) [Found: (micro.) C, 67·0; H, 9·9; N, 17·0, 16·8; *M*, 273. Calc. for $C_{14}H_{25}ON_3$: C, 66·9; H, 10·0; N, 16·7%; *M*, 251].

Methylheptenone. The portion insoluble in the light petroleum was dissolved in benzene, from which solution it separated as a colourless microcrystalline solid, m. p. 129—131°, on addition of light petroleum. The properties of this semicarbazone are in fair agreement with those given in the literature (Tiemann and Kruger, *Ber.*, 1895, **28**, 2124) for methylheptenone semicarbazone, which is described as a colourless solid, m. p. 136—138°, difficultly soluble in light petroleum and soluble in benzene. Unfortunately, insufficiency of material precluded further purification and more definite characterisation (Found: N, 22·6. Calc. for $C_9H_{17}ON_3$: N, 22·9%).

Action of Chromyl Chloride upon Squalene.—A 10% solution of chromyl chloride (12 mols.) in dry carbon disulphide was added in small portions at a time and with vigorous shaking to a solution of squalene (24 g.) in the same solvent. The reaction, which was completed in 7 hours, was carried out at −15°. The resultant dark brownish-green precipitate was repeatedly washed with dry carbon disulphide. The filtrate was practically free from chromyl chloride. The addition compound was decomposed with iced water, and the aqueous liquor distilled from a steam-bath, a fraction, b. p. 60—80°, being collected and gaseous material absorbed in water. The

neutral aqueous solution obtained showed strong reducing properties and the presence of formaldehyde was readily detected by means of the Schryver reagent. The solution was carefully oxidised with potassium permanganate (0·5% solution) and evaporated to dryness. On extraction of the resultant solid with hot absolute alcohol, potassium acetate was removed, thus proving that acetaldehyde must also have been present in the oxidised material. An examination of the fraction of b. p. 60—80° revealed the presence of acetone, which was isolated both as the *p*-nitrophenylhydrazone (m. p. 148°) and as the semicarbazone (m. p. 187°).

The residue in the flask was separated into acid and neutral portions. From the former, a small amount of succinic acid was obtained; the latter, which consisted of a heavy resinous oil, resisted all attempts at purification.

Squalene Dodecabromide.—A solution of squalene (20 g.) in sodium-dried ether (150 c.c.) was treated at −25° with a solution of dry bromine in ether (90 g. in 200 c.c.). In 30 minutes a colourless crystalline solid separated which, after drying, darkened at 160° and melted at 185° (decomp.). A further crop was obtained by evaporating the filtrate under reduced pressure and triturating the resultant sticky yellow solid with ethyl acetate (total yield, 80%). The bromide can also be prepared directly from the crude liver oil, the product being somewhat darker in colour, but the total yield remains about the same.

Analysis of the crude product. (A) From squalene obtained from *Centrophorus granulosus*: Found, Br, 69·5%. (B) From *Scymnorhinus licia*: Found, C, 26·8, 26·8; H, 3·6, 3·8% ($C_{28}H_{46}Br_{12}$ requires C, 25·0; H, 3·4; Br, 71·6%. $C_{29}H_{48}Br_{12}$ requires C, 25·7; H, 3·5; Br, 70·8%. $C_{30}H_{50}Br_{12}$ requires C, 26·3; H, 3·6; Br, 70·1%. $C_{31}H_{52}Br_{12}$ requires C, 26·9; H, 3·8; Br, 69·3%).

Isolation of the bromide, m. p. 198°. The crude bromide (30 g.) was boiled with ethyl acetate (200 g.), and the soluble portion removed by rapid filtration under pressure. The insoluble residue was repeatedly washed with boiling ethyl acetate until a sample of the filtrate left no deposit on evaporation. The dodecabromide formed a hard, microcrystalline, colourless solid, m. p. 198° (decomp. but without previous darkening), insoluble in the common organic solvents but soluble to some extent in pyridine, tetrachloroethane and xylene [Found (squalene from *Centrophorus granulosus*): C, 26·2, 26·3; H, 3·4, 3·4; Br, 69·9%. Found (squalene from *Scymnorhinus lichia*): C, 26·4, 26·4; H, 3·7, 3·8%].

Soluble bromide. The brown ethyl acetate filtrate deposited on cooling a solid, m. p. 178°, which decomposed to a marked extent in moist air with a lowering of m. p. to 174° (unsharp). Attempts

to purify this were unsuccessful owing to obvious decomposition in the boiling solvent (Found on immediate analysis: C, 26·7; H, 3·8; Br, 69·5%. Found after 14 days: C, 28·5; H, 3·8%).

In conclusion we desire to express our thanks to Mr. E. B. Parkes, M.Sc., for help in the examination of the squalene dodecabromide, and to the Food Investigation Board for a grant which has enabled this investigation to be carried out.

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