CCXIII.—*The Unsaponifiable Matter from the Oils of Elasmobranch Fish. Part I. A Contribution to the Study of the Constitution of Squalene (Spinacene).*

By Isidor Morris Heilbron, Edward David Kamm, and
William Morgan Owens.

The first mention of the occurrence of squalene in fish-liver oils is due to Tsujimoto (*J. Chem. Ind. Tokio*, 1906, **9**, 953), who subsequently described its physical characteristics and assigned to it the molecular formula $C_{30}H_{50}$ (*J. Ind. Eng. Chem.*, 1916, **8**, 889; 1920, **12**, 63). The hydrocarbon was found to yield a well-defined crystalline hexahydrochloride, but of indefinite melting point, sintering at about 112° and finally melting to a clear liquid at about 125°. Meanwhile, in 1915, A. Chaston Chapman, working independently, isolated from fish-liver oils obtained from Portugal what would appear to be the same hydrocarbon, for which he proposed the name spinacene, and assigned to it the same molecular formula (J., 1917, **111**, 56). Later (J., 1918, **113**, 458), from the results of other analyses of the hexahydrochloride and hexahydrobromide and of molecular-weight determinations, he concluded that its constitution was in better accord with the formula $C_{29}H_{48}$. This author also remarked on the indefinite nature of the melting point of the hydrochloride. He found that whereas the crude compound commenced to shrink at 100° and began to melt at 110° (Cl, 33·8%),* after purification by means of benzene and alcohol, the final preparation became pasty at 120° and melted to a clear liquid at 126° (Cl, 34·5%). In a recent communication by André and Canal (*Compt. rend.*, 1925, **181**, 612), an account is given of the hydrochlorides prepared from the unsaponifiable matter of the oils of *Cetorhinus maximus* (A) and *Scymnorhinus lichia* (B). Whilst the oils from both fish had previously been examined by Tsujimoto and found to contain squalene, that from the latter has been used by us and was also almost certainly employed by Chapman (*Analyst*, May, 1917). From A, André and Canal separated two hydrochlorides: A_1, m. p. 107—108° (Cl, 35·3%, corresponding with a hydrocarbon $C_{28}H_{46}$); and A_2, m. p. 144—145° (Cl, 33·9%, indicating $C_{30}H_{50}$). On the other hand, they obtained from B the hydrochlorides B_1, m. p. 107—108° (Cl, 33·9%), and B_2, m. p. 143—145° (Cl, 33·2%), the latter chlorine content corresponding to the hydrocarbon $C_{31}H_{52}$. A mixture of A_1 and B_1 is stated to show no depression of melting point, whilst A_2 and B_2 give a slight depression (139—140°). From these highly conflicting results, the authors claim that neither squalene nor spinacene is a definite chemical compound, but each is a mixture of various unsaturated hydrocarbons from which the $C_{29}H_{48}$ homologue proposed by Chapman is apparently absent. We agree with André and Canal in so far that we have separated hydrochlorides of different melting points, but find for each *the same values* for carbon, hydrogen, and chlorine in strict agreement with a hydrocarbon formula $C_{30}H_{50}$.† It would appear that three isomeric hydrochlorides, m. p. 107—108°, 113—114°, and 144—145°, exist, although it is somewhat doubtful whether the lowest-melting one is a pure compound (see experimental part). The hydrocarbon has been regenerated from each hydrochloride and reconverted into its hydrochloride, which, in every case, is again separable into low- and high-melting fractions.

	C %.	H %.	Cl %.
* $C_{28}H_{54}Cl_6$	55·9	8·7	35·4
$C_{29}H_{54}Cl_6$	56·7	8·8	34·6
$C_{30}H_{56}Cl_6$	57·2	8·9	33·9
$C_{31}H_{58}Cl_6$	57·9	9·0	33·1

[†] The squalene used was obtained from *Lepidorhinus squamosus*, *Etmopterus spinax*, *Centrophorus granulosus*, and *Scymnorhinus lichia*, and the material from each source gave the same results.

TABLE I.

The Constants of Squalene.

	Chapman.	Tsujimoto.	Majima and Kubota.	Present authors.
B. p. at 0·15 mm.			205°	
0·55 „			235—237°	
2 „				240—242°
4 „				248—250°
5 „		252—254°		
9 „	260°			261—262°
10 „		262—264°		
15 „				270—275°
25 „			284—285°	
Density	0·8610/$^{15°}_{15°}$		0·8596/$^{15°}_{4°}$	0·8655/$^{13°}_{13°}$
	0·8588/$^{20°}_{20°}$	0·8587/$^{15°}_{4°}$	0·8584/$^{20°}_{4°}$	0·8538/$^{20°}_{20°}$
	0·8616/$^{20°}_{4°}$			0·8596/$^{18°}_{18°}$
Refractive index $n_D^{15°}$ ...	1·4987		1·4959, 1·4966	
$n_D^{18°}$ ...				1·4972
$n_D^{20°}$ ...	1·4967, 1·4951	1·4965	1·4965	1·4965, 1·4980
Mol. refraction	139·1, 134·5*	139·1	139·8	139·6, 139·8, 139·9
Iodine value	367·9			377·6
Mol. wt. (cryoscopic)	394, 391 (benzene)	408 (benzene)	407 (ethylene dibromide)	391 (benzene) 439 (ethylene dibromide)

* Calculated for $C_{28}H_{48}$.

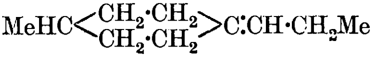
These results lead to the conclusion, which is also borne out by other evidence, that squalene and spinacene are one and the same hydrocarbon of the molecular formula $C_{30}H_{50}$. Taking into account the number of species of fish from which squalene has been isolated, we are of the opinion that no evidence has been adduced in favour of André and Canal's view (*loc. cit.*) that homologues of this hydrocarbon exist in marine animal oils. On the other hand, the isolation of the isomeric hydrochlorides would appear to indicate that squalene is itself a mixture of isomerides. In support of this view, it has repeatedly been found that the density and refractive index are not absolutely constant, although the calculated molecular refraction is almost exactly the same for different specimens. This value shows a marked exaltation ($\Sigma[R_L]_D = 1{\cdot}8—2{\cdot}3$) over the theoretical value for $C_{30}H_{50}|\overline{6}$. This exaltation is not due, however, to the presence, as André and Canal claim, of a $C_{31}H_{52}$ homologue in squalene, for the hydrocarbon regenerated from the hexahydrochloride melting at 107—108°, which, according to these authors, corresponds to $C_{30}H_{50}$ in the case of *Scymnorhinus lichia*, also shows exactly the same exaltation. The exaltation is apparently not due to the presence of conjugated double bonds, since we, confirming Majima and Kubota (*Jap. J. Chem.*, 1922, **1**, 9), have found that squalene is inert towards sodium and boiling amyl alcohol.

Sources of Squalene.

Squalene is widely distributed among elasmobranchs. It occurs chiefly in the family *Squalidæ*, but is not found in every member of it. Moreover, it is not peculiar to elasmobranchs, for Drummond, Channon, and Coward (*Biochem. J.*, 1925, **19**, 1047) have reported its presence in the unsaponifiable matter of cod-liver oil. The hydrocarbon, although occurring chiefly in the liver oil, has also been found in the egg oils of *Chlamedoselachus anguineus* and *Lepidorhinus kinbei* by Tsujimoto (*loc. cit.*), and of *Etmopterus spinax*, *L. squamosus*, and *Scymnorhinus lichia* by us. Moreover, the intestines of the last-mentioned fish always contain a large amount (up to 300 c.c.) of a dark oil, the greater part of which is squalene. This may be produced *post-mortem*—the specimens when treated have always been dead for several days—but the fact that it is invariably present seems to us to favour the view that it is a characteristic of the living animal. An examination was made in May, 1925, of 115 female *E. spinax*. From some of these, eggs in a comparatively undeveloped condition were dissected, all of which contained squalene. In others, almost completely developed embryo with yolk-sac were found. The sac did not contain the hydrocarbon, which must therefore have been absorbed during the growth of the embryo. A noteworthy fact regarding squalene-containing fish is the remarkable health of their bodies, in which internal parasites are very rare (private communication from Professor Johnstone).

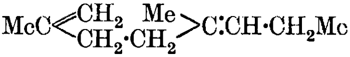
Decomposition of Squalene by Heat.

This has been studied by Chapman (J., 1923, **123**, 769) and Majima and Kubota (*loc. cit.*). The latter investigators subjected squalene to dry distillation and obtained fractions boiling at all temperatures from 36°/760 mm. to 235°/18 mm. They claim to have identified isoprene; the constants recorded, however, are very low ($D_{4^\circ}^{18^\circ}$ 0·6692, $n_{D}^{18^\circ}$ 1·39641; compare Harries, *Ber.*, 1914, **47**, 1999). From the most abundant higher fraction, a liquid, $C_{10}H_{18}$ (b. p. 62·5—66°/17 mm.), was obtained the physical constants of which resemble those of *cyclo*dihydromyrcene or *cyclo*dihydrolinolo-olene. Chapman's methods of decomposition were somewhat different, but gave apparently similar products. He concludes that the low-boiling fraction is a mixture of one or more amylenes with some isoprene or isomeric substance, whilst the main products are considered to be 1-methyl-4-propylidene*cyclo*hexane (I) and the closely related diolefinic hydrocarbon (II).



$$\mathrm{MeHC}{<}\begin{matrix}\mathrm{CH_2{\cdot}CH_2}\\\mathrm{CH_2{\cdot}CH_2}\end{matrix}{>}\mathrm{C{:}CH{\cdot}CH_2Me}$$

(I.)



$$\mathrm{MeC}{<}\begin{matrix}\mathrm{CH_2\ \ Me}\\\mathrm{CH_2{\cdot}CH_2}\end{matrix}{>}\mathrm{C{:}CH{\cdot}CH_2Me}$$

(II.)

By the dry distillation of squalene and careful fractionation of the product, we obtained members not only of the hemiterpene and monoterpene groups, but also of the sesquiterpene, diterpene, and probably even higher divisions of the same family.

The lowest-boiling liquid member obtained by us has been definitely proved to be an amylene, oxidation of which by potassium permanganate yielded acetone, from which it follows that its structure must be $CMe_2{:}CHMe$ (III).

The fraction of next higher boiling point (62—100°/20 mm.), which appears to be a mixture of monoterpenes and dihydromonoterpenes, is under investigation.

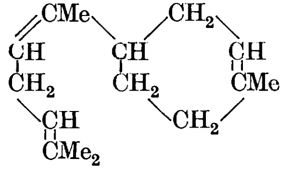
The third fraction, boiling between 120° and 170°/20 mm., consists of a complex mixture of monocyclic sesquiterpenes. By repeated distillation a constant-boiling fraction has been isolated the physical characteristics of which (Table II) are identical with those of bisabolene (Ruzicka, *Helv. Chim. Acta*, 1925, **8**, 259). In addition, ring closure by means of formic acid has yielded a dicyclic isomeride the constants of which agree closely with those of the dicyclic sesquiterpenes isolated by Ruzicka from nerolidol and which, according to this author, could only have resulted through the intermediate formation of farnesene and bisabolene (Table II).

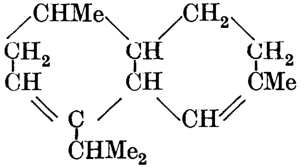
TABLE II.

	B. p./12 mm.	$d_{21°}^{21°}$.	$d_{4°}^{21°}$.	$n_D^{21°}$.	$[R_L]_D$.
Hydrocarbon from squalene ...	129—135°	0·8734	0·8716	1·4915	67·72
Synthetic bisabolene	130—137	—	0·8717	1·4923	67·99
					(Calc., 67·87)

In an attempt to obtain further evidence regarding the carbon skeleton of the entire sesquiterpene fraction, this was dehydrogenated with sulphur (compare Ruzicka, *Helv. Chim. Acta*, 1922, **5**, 345), and the product distilled over sodium. In three different experiments, addition of alcoholic picric acid produced, in insufficient quantity for analysis, orange crystals of what appeared from the melting point to be cadalene picrate. The difficulty of obtaining cadalene in appreciable quantity is not peculiar to the sesquiterpene mixture handled by us, for Staudinger (*Helv. Chim. Acta*, 1922, **5**, 785) failed to isolate any trace of this hydrocarbon on dehydrogenation of the sesquiterpene fraction from caoutchouc, which should most certainly have yielded it. Moreover, Ruzicka was unable to obtain cadalene from the compound (IV) (*Helv. Chim. Acta*, 1923, **6**, 487), whereas it was readily obtained from cadinene (V), which is similarly constituted.

(IV.)





(V.)

From the complex mixture of higher-boiling fractions dicyclic diterpenes have been isolated, and these, on treatment with formic acid, pass into tricyclic isomerides, a reaction reminiscent of the conversion of the monocyclic α-camphorene into its tricyclic isomeride (Ruzicka, *Helv. Chim. Acta*, 1924, **7**, 279). The residue, which probably consists of members of the next higher group ($C_{25}H_{40}$), has not yet been examined.

Isomerisation of Squalene.

In addition to the isomerism due to variation in position of one or more of the ethenoid linkings, there exists, as might have been anticipated, isomerism due to ring formation. Majima and Kubota (*loc. cit.*) were the first to prove that squalene can be so isomerised, but it is apparent from their results that they failed to obtain a pure isomeride. As shown in Table III, 95% formic acid is the best catalyst for effecting ring closure in the squalene molecule, which, we have found, cannot be cyclised beyond the stage at which two double bonds remain (Table III).

TABLE III.

Molecular Refractions of Squalene Isomerides.

Isomerising agent.	Time in hours.	Ethenoid linkings (iodine values).	$[R_L]_D$. Found.	$[R_L]_D$. Calc.	$\Sigma[R_L]_D$.
(Pure squalene)	—	6	139·8	137·98	1·85
Acetic anhydride with sulphuric acid	$2\frac{1}{2}$	—	136·9	—	—
Alcoholic sulphuric acid ...	18	—	134·0	—	—
Formic acid	$\frac{1}{2}$	—	136·9	—	—
,, ,, ...	3	3	134·3	132·6	1·7
,, ,, ...	9	3	134·1	132·6	1·5
,, ,, ...	24 (or longer)	2	133·2	130·9	2·3

Constitution of Squalene.

At this point we would again emphasise our conviction that squalene and spinacene are identical. In addition to the evidence of the hydrochloride formation, we are led to this conclusion by the following considerations :

(*a*) The physical properties of spinacene as recorded by Chapman agree closely with those recorded by all other workers in the same field (Table I).

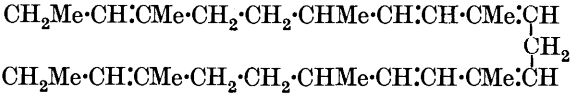
(*b*) The calculated molecular refraction for $C_{29}H_{48}|^{=}_{6}$ is 133·1, not 134·3 as stated by Chapman (J., 1918, **113**, 458). The value of 134·5 determined by him is in no better agreement with this than the value worked out from the same data for $C_{30}H_{50}|^{=}_{6}$ (139·3) is with the theoretical value 137·7.

(*c*) It does not appear to us that evidence based on cryoscopic measurements can be considered sufficiently trustworthy to distinguish between successive homologues ($C_{29}H_{48}$,$C_{30}H_{50}$) of such high molecular weight.

(*d*) The recorded chemical properties of squalene and spinacene are identical.

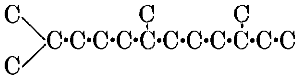
The constitution of spinacene (VI) tentatively proposed by Chapman will therefore not be discussed, not only because of the difficulty of accepting the $C_{29}H_{48}$ molecular formula, but because it fails to account for the formation of the typical terpene products above mentioned.

(VI.)



$$\begin{array}{r} CH_2Me\cdot CH{:}CMe\cdot CH_2\cdot CH_2\cdot CHMe\cdot CH{:}CH\cdot CMe{:}CH \\ | \\ CH_2 \\ | \\ CH_2Me\cdot CH{:}CMe\cdot CH_2\cdot CH_2\cdot CHMe\cdot CH{:}CH\cdot CMe{:}CH \end{array}$$

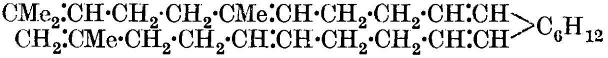
That squalene is a terpene compound is evidenced by the ease with which it undergoes ring closure, by its ready regeneration from the hexahydrochloride, and by the large quantities of succinic and lævulic acids obtained on decomposition of its ozonide (Majima and Kubota, *loc. cit.*). The isolation of bisabolene proves that at least one-half of the squalene molecule must have the carbon skeleton



$$\left.\begin{array}{l} C \\ C \end{array}\right> C\cdot C\cdot C\cdot C\cdot \overset{C}{C}\cdot C\cdot C\cdot C\cdot \overset{C}{C}\cdot C\cdot C$$

and the fact that diterpenes have also been obtained indicates that, as suggested by Majima and Kubota, the hydrocarbon is a dihydrotriterpene. We do not agree, however, with the structure (VII) postulated by these authors, which fails to show the complete relationship of squalene to terpene hydrocarbons in general, but consider that squalene is an equilibrium mixture of isomerides, one form of which might be represented by (VIII).

(VII.)



$$\left.\begin{array}{l} CMe_2{:}CH\cdot CH_2\cdot CH_2\cdot CMe{:}CH\cdot CH_2\cdot CH_2\cdot CH{:}CH \\ CH_2{:}CMe\cdot CH_2\cdot CH_2\cdot CH{:}CH\cdot CH_2\cdot CH_2\cdot CH{:}CH \end{array}\right> C_6H_{12}$$

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

Table IV shows that, as is to be anticipated from formula (VIII), the closest analogy exists between the products of decomposition of squalene and caoutchouc (Staudinger and Fritschi, *Helv. Chim. Acta*, 1922, **5**, 785).

Significance of Squalene.

That squalene is intimately connected with metabolic processes is self-evident, and in this connexion we desire to direct attention to a possible relationship between this hydrocarbon, stigmasterol ($C_{30}H_{50}O$), and cholesterol ($C_{27}H_{46}O$).* Feeding experiments carried out on rats by Mr. H. J. Channon, of University College, London,

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* [Note added, June 18th.] Attention is also directed to the recent paper of Oikawa (*J. Biochem.* [*Japan*], 1925, **5**, 63), the abstract of which has just appeared, in which the isolation from shark bile of a pentahydric alcohol, $C_{27}H_{48}O_5,2H_2O$, is reported.

show that the administration of squalene results in the amount of cholesterol in the liver being more than doubled. This observation may support the view that squalene is a precursor in cholesterol synthesis in the animal body; on the other hand, the large cholesterol increase may be due to some factor yet to be determined. Further biochemical work is in progress to confirm or disprove the hypothesis.

TABLE IV.

Comparison of the Products of Decomposition of Squalene and Caoutchouc.

Product.	Source.	*M.*	B. p.	*d.*	n_D.	$[R_L]_D$.	$[R_L]_D$ calc. for
C_5H_8	Caoutchouc		30—50°/760 mm.				
C_5H_{10}	Squalene		32—40°/760 mm.				
$C_{10}H_{16}$	Caoutchouc	142	58°/11 mm.	0·8390/20°	1·4724/20°	45·42	$C_{10}H_{16}\|_{2}^{=}$
	Squalene (cyclised by HCO_2H)		86—101°/25 mm.	0·8326/21°	1·465/21°	45·17	= 45·24
$C_{15}H_{24}$	Caoutchouc	200	88—91°/0·05 mm.	0·8895/20°	1·4980/20°	67·23	$C_{15}H_{24}\|_{3}^{=}$
	Squalene		129—135°/12 mm.	0·8734/21°	1·4915/21°	67·8	= 67·83
			152—162°/15 mm.	0·8934/16°	1·499/16°	67·04	
$C_{20}H_{32}$	Caoutchouc	268	118—122°/0·02 mm.	0·9046/20°	1·5063/20°	89·41	$C_{20}H_{32}\|_{3}^{=}$
	Squalene		180—190°/9 mm.	0·9073/19°	1·5080/19°	89·33	= 88·7
		259	190—200°/9 mm.	0·9157/19°	1·513/19°	88·88	
	Caoutchouc	303	142—148°/0·04 mm.	0·9161/20°	1·5119/20°	89·17 (if $C_{20}H_{32}$) 111·2 (if $C_{25}H_{40}$)	$C_{25}H_{40}\|_{4}^{=}$ = 111·3

EXPERIMENTAL.

Extraction of Oils.—The fish were dissected immediately on arrival, and the livers removed and weighed. The stomach was in each case ligatured and the contents were examined for the presence of oil. Any eggs in the females were separated and examined. Table V gives the oil content of the various species. The livers were cut into small pieces and the oil was extracted by heating with a coil into which steam was admitted for about ½ hour. The liberated oil was separated from tissue matter by means of a centrifuge and was obtained as a clear mobile liquid, pale yellow to orange in colour.

Extraction of the Hydrocarbon from the Liver Oil.—(a) *Saponification.* Considerable difficulty was experienced in saponifying the oil and separating the unsaponifiable matter. After many experiments, including saponifications in alcohol, it was found that the most satisfactory method was to heat the oil (500 c.c.) in a nickel basin to 105—110° and add drop by drop an equal volume of 20% sodium hydroxide solution with continuous stirring. After saponification was complete (about 1 hour), sodium chloride (150 g.) was added together with 150 c.c. of hot water. The mixture when cool was filtered through cloth, and the oil was separated from the aqueous layer, dried over sodium sulphate, and distilled at 3 mm. pressure. The squalene so obtained had a refractive index of about 1·497 and contained a small quantity of oxygenated substance (compare Chapman, J., 1917, **111**, 56).

TABLE V.

Measurements of Fish and Yield of Oil.

Fish.	Date.	No.	Sex and condition.	Length (cm.).	Wt. (g.).	Wt. of liver (g.).	Liver oil (c.c.).	Stomach oil (c.c.).	Squalene in liver oil (%).
Scymnorhinus lichia.	12.3.24	1		113	7190	1530	2000	200	Average = 55
		1		112	6270	1542			
	8.5.25	1	Male.	101	4550	840	800	150	
	12.5.25	1	Male.	125	—	2230	2000	250	
	20.5.25	5	Males.	Average = 110	—	—	1230	130	
		1	Female.	100	—	—	1000	100	
Etmopterus spinax.	11.5.25	115	Mature females.	Average = 23	108	30	18	—	50
Centrophorus granulosus.	29.5.25	1	Female (after spawning).	131	—	2950	2500	—	80
Lepidorhinus squamosus.	1.11.24	1	Mature female.	125	—	3800	3000	—	65
		1	Female (after spawning).	123	—	1800	1500	—	
	1.5.25	5	Males.	Average = 109	—	1570	750	—	74

(b) *Distillation Method.*—This method (compare Tsujimoto, *loc. cit.*) is more rapid than the preceding for the preparation of large quantities of the hydrocarbon. The oil (in portions of not more than 200 g.) was distilled under 3 mm. pressure; no distillate appeared below 235°, the major portion distilling regularly between 240° and 250°. Distillation was stopped as soon as the first signs of fuming were noticed, and an oil, containing as chief impurity a small quantity of free fatty acid, was thus obtained ($n_{\mathrm{D}}^{20^\circ}$ 1·497—1·498). It was dissolved in ether, the solution, after being washed several times with dilute sodium hydroxide solution and finally with water, was dried over calcium chloride, the ether removed, and the residual oil distilled. The products ($n_{\mathrm{D}}^{20^\circ}$ 1·498) thus obtained from different samples, as in the previous case, contained varying quantities of oxygen (C, 87·4, 87·2; H, 11·9, 12·0%). Pure squalene can, however, be obtained from them (*a*) through the hydrochloride, (*b*) by treatment with phthalic anhydride.

The crude hydrocarbon (160 g.) was heated for 5 hours with phthalic anhydride (18 g.) at 130—140°. After cooling and removal of unchanged anhydride, the oil in ethereal solution was shaken successively with dilute sodium carbonate and sodium hydroxide solution and washed with water. The ether was removed from the dried solution, and the residual oil distilled, the whole coming over at 260—262°/9 mm.; $n_{\mathrm{D}}^{18^\circ}$ 1·4982, $n_{\mathrm{D}}^{20^\circ}$ 1·4965, $d_{18^\circ}^{18^\circ}$ 0·8596; $[R_L]_{\mathrm{D}}$ 139·6 (Found: C, 87·7, 87·7; H, 12·1, 12·0. $C_{30}H_{50}$ requires C, 87·8; H, 12·2%).

The sodium carbonate washings contained no acid phthalate, and it must be concluded that the impurity present in the crude hydrocarbon is either a tertiary alcohol, which would seem to be closely related to the hydrocarbon, or an internal oxide similar in nature to cineole, which compound on treatment with dehydrating agents readily passes into dipentene. This point is being further examined.

Treatment of crude squalene with acetic anhydride and *d*-camphorsulphonic acid also removes the impurity as a lower-boiling liquid of sharp odour. This method, however, cannot be used for the preparation of squalene, because isomerisation also occurs.

Squalene Hexahydrochloride. General Method of Preparation.—Dry acetone (15 g.) was saturated with dry hydrogen chloride at —5° and squalene (5 g.) was added, the stream of gas being continued until the whole became solid; the colour of the solution changed through red to almost black. The resulting hydrochloride (6 g.) was washed free from coloured oily impurity with dry ether. By keeping the filtrate, again saturated with hydrogen chloride, in the ice-chest, a further small crop was obtained (approx. 3%). If the acetone is not presaturated with hydrogen chloride, the first crop obtained is very much smaller; the total yield, however, is only slightly diminished, as a large crop separates during the second treatment. This method is much slower and generally less satisfactory than the first. Ether also can be employed as solvent, but cannot be relied upon to give such consistent yields, being apparently sensitive to minute alteration of conditions. Analysis of the washed crude hydrochloride from any of the sources examined by us always gave similar analytical values (*e.g.*, found: C, 57·1, 57·0; H, 9·0, 8·8%). The hydrochloride can be prepared equally readily from squalene-containing oils.

Separation of the Isomerides. Isolation of the 107—108° *Hydrochloride from Squalene obtained from* Scymnorhinus lichia.—The crude hydrochloride (14 g.), m. p. 109—112°, was digested with dry acetone (300 c.c.) for 10 minutes at 50°, boiled for one minute, and the residue (2·4 g.) filtered off. The filtrate on standing deposited hydrochloride (9·2 g.) which melted at 107—108° (Found: C, 57·2, 57·2; H, 8·8, 9·1; Cl, 33·6%). The yields of this compound, which was not always produced, varied widely even when the conditions were precisely similar. Although it appeared to be a definite isomeride, nevertheless, on digestion with hot acetone, a small residue (m. p. 111—122°) was invariably obtained.

Isolation of the High-melting Isomeride.—The residue (m. p. 126—130°) obtained above was combined with other sparingly soluble fractions which had been worked up in the same way. The total material (6 g.) was boiled with acetone (175 c.c.), which was then filtered quickly. The undissolved solid (2·3 g.), m. p. 140—142°, was again similarly treated with acetone (250 c.c.), and the residue (1·7 g.), now melting at about 144°, recrystallised from ethyl acetate. It formed characteristic rhombic plates, m. p. 144—145°, very similar in appearance to the 107—108° isomeride, and was practically insoluble in acetone or alcohol and moderately easily soluble in ethyl acetate (Found: C, 56·9, 56·8; H, 8·9, 8·8; Cl, 33·6, 33·8%). The intermediate portions obtained from the filtrates melted in-definitely between 111° and 120° and were separated into the high-melting hydrochloride and an isomeride, m. p. 113—114°.

The latter compound was readily prepared as follows: The washed crude hydrochloride (36 g.) was recrystallised from ethyl acetate (270 c.c.), and the separated solid (32 g., m. p. 111—120°) digested with boiling ethyl acetate. The insoluble residue (11 g., m. p. 117—130°) was now taken up in hot acetone and separated from 5 g. of undissolved material (m. p. 131—137°) by filtration. From the filtrate, crystals, m. p. 113—114°, were obtained. This compound showed no alteration in melting point on further crystallisation from acetone, in which it is moderately easily soluble, although less so than the 107—108° form (Found: C, 56·8; H, 8·9; Cl, 33·8, 33·9%).

Regeneration of Squalene from the Hydrochlorides.—(A) 113—114° *Isomeride.* The hydrochloride (17 g.) was boiled under reflux with pyridine (68 g.) for 4 hours in an atmosphere of carbon dioxide. The reaction mixture was poured into an excess of dilute sulphuric acid, and the hydrocarbon extracted with ether. The ethereal solution was well washed with water, dried, and, after removal of the solvent, the residual oil was distilled at 4 mm. The constant-boiling fraction was again redistilled at the same pressure, and the major portion, boiling at 240—242°, collected separately, $n_D^{20°}$ 1·4990, $d_{20°}^{20°}$ 0·8592, $[R_L]_D$ 140·2, $[R_L]_D$ calc. for $C_{30}H_{50}|_{\overline{6}}^{=}$ 137·7 (Found: C, 87·8, 87·5; H, 12·1, 12·2. $C_{30}H_{50}$ requires C, 87·8; H, 12·2%). The regeneration can also be brought about with alcoholic sodium ethoxide, silver acetate, or quinoline. Even prolonged boiling with absolute alcohol is sufficient to effect complete decomposition of the hydrochloride.

(B) 107—108° *Isomeride.* This was similarly decomposed and the constants of the pure hydrocarbon were determined, $n_D^{20°}$ 1·4955, $d_{20°}^{20°}$ 0·8563, $[R_L]_D$ 139·8 (Found: C, 87·6, 87·7; H, 12·3, 12·3%).

(C) 144—145° *Isomeride.* This proved slightly more difficult to decompose. The constants of the hydrocarbon after distillation over sodium were: $n_D^{16·5°}$ 1·4990, $d_{16·5°}^{16·5°}$ 0·8612, $[R_L]_D$ 139·8 (Found: C, 87·4; H, 12·2%).

Regeneration of the Hydrochlorides.—Each of the regenerated hydrocarbons was converted into the hydrochlorides in the usual way and these, on treatment with acetone as previously described, were separated into high- and low-melting components.

Treatment of the Oily Residues.—After removal of the major portions of the hydrochloride, the acetone and ethyl acetate mother-liquors invariably yielded, on concentration, successive crops of lower-melting compounds (*e.g.*, 95—96°, 84°, and 69°) and finally a sparingly soluble oil separated. That this consisted of partly decomposed hydrochloride was shown by again saturating it with hydrogen chloride, when, on standing at 0°, the usual mixture of isomerides (m. p. 110—116°) was again produced (Found : C, 57·0, 57·1 ; H, 8·9, 8·9%).

Squalene Hexahydrobromide.—Tsujimoto (*loc. cit.*) gives m. p. 115—126° for this compound, whilst Chapman states that, after three crystallisations from benzene, it softens at 126° and melts to a clear liquid at about 132°. Provided that the two compounds are identical, a similar discrepancy occurs in the analytical values, the results obtained by the former (C, 40·2 ; H, 6·4 ; Br, 53·0, 53·5%) being in good accord with $C_{30}H_{50}$, whilst Chapman's result (Br, 54·5%) agrees with $C_{29}H_{48}$. We have prepared this compound by the usual method (5 g. of squalene) and obtained it by recrystallisation from hot acetone in lustrous plates melting at 118—120° to a clear liquid. Although this compound has not been prepared in large quantity, a sufficient examination has been made to show that higher-melting isomerides can be obtained. The m. p. of a specimen that had been kept for 2 months in a desiccator over solid sodium hydroxide fell to 110—113°, doubtless owing to partial decomposition (Found : C, 40·1 ; H, 6·3 ; Br, 53·5, 53·7. $C_{30}H_{56}Br_6$ requires C, 40·2 ; H, 6·3 ; Br, 53·5%).

Isomerisation.—(a) *Acetic anhydride containing sulphuric acid.* Squalene (150 g.) was boiled under reflux for 2½ hours with acetic anhydride (200 c.c.) containing 2 c.c. of sulphuric acid. The product was poured into water, neutralised with sodium carbonate, and extracted with ether. After removal of solvent from the dried solution, distillation of the residue yielded a pale yellow oil, b. p. 253—256°/4 mm., $n_D^{20°}$ 1·5080, $d_4^{20°}$ 0·8939, $[R_L]_D$ 136·7.

(b) *Alcoholic sulphuric acid.* Squalene (10 g.) was boiled under reflux with 50 c.c. of alcoholic sulphuric acid (15% H_2SO_4) for 2 days. The product, after extraction as above, gave on distillation a main fraction, b. p. 235—240°/3 mm., $n_D^{15°}$ 1·5049, $d_{15°}^{15°}$ 0·9073, $[R_L]_D$ 134·01.

(c) *Formic acid.* The hydrocarbon was boiled under reflux with twice its weight of formic acid (98%) for various periods. After cooling, the solution was diluted with water and treated as above. The products were :

Reaction period, ½ hour.—Mobile, pale yellow oil, b. p. 215—225°/2 mm., $n_D^{15°}$ 1·5060, $d_{15°}^{15°}$ 0·8900, $[R_L]_D$ 136·8.

Reaction period, 3 *hours.*—Rather viscous, pale yellow oil, b. p. 232—233°/3 mm., $n_D^{15°}$ 1·5150, $d_{15°}^{15°}$ 0·9207, $[R_L]_D$ 134·3, iodine value 179·9 ($C_{30}H_{50}|\overline{\overline{3}}$ requires iodine value 185·8, $[R_L]_D$ 132·6).

Reaction period, 24 *hours to* 3 *days.*—Very viscous, pale yellow oil, b. p. 230—232°/3 mm., $n_D^{15°}$ 1·5211, $d_{15°}^{15°}$ 0·9359, $[R_L]_D$ 133·2, iodine value 117·4 ($C_{30}H_{50}|\overline{\overline{2}}$ requires iodine value 124·6, $[R_L]_D$ 130·9).

It will be seen that the isomerisation occurs in stages. On the assumption, which seems likely from the final results, that the exaltation of the molecular refraction persists throughout, the product of $\frac{1}{2}$ hour's treatment contains two rings, that of 3 hours' treatment, three rings, whilst the maximum ring closure (four) is reached after 3 days, when only two double bonds remain. For each ring formed, an increase in density and in viscosity occurs; the final product has the consistency of thick treacle, only pouring with difficulty from a test-tube at room temperature.

Attempted Reduction of Squalene by Sodium and Amyl Alcohol.—A mixture containing squalene (5 g.), sodium (10 g.), and amyl alcohol (20 g.) was heated to boiling, and to this, amyl alcohol was added drop by drop until all the sodium had dissolved. After treatment with water, and extraction with ether, the whole was distilled; unchanged squalene was then recovered ($n_D^{13^\circ}$ 1·5022, $d_{13^\circ}^{13^\circ}$ 0·8655, $[R_L]_D$ 139·85). The reaction was repeated with *n*-octyl alcohol, with the same result.

Dry Distillation of Squalene.—Squalene (700 g.) was distilled, in portions of not more than 150 c.c., from a 250 c.c. flask through an air condenser into another distillation flask, which served as a receiver for all high-boiling liquid. The side tube of this receiver was connected to a spiral of glass tube immersed in a freezing mixture of ice and salt, which effectively condensed the low-boiling products of the reaction. The outlet of this spiral was connected to an absorption bottle containing a 10% solution of bromine in chloroform.

During the distillation, no appreciable absorption took place, although a small quantity of a combustible gas passed through the bromine solution. The total distillate amounted to about 600 g., the residue being a polymerised resin.

The distillate was separated by fractionation into five main portions :

	B. p.	Pressure.	Wt.		B. p.	Pressure.	Wt.
1.	30—45°	760 mm.	36 g.	4.	170—20°	115 mm.	110 g.
2.	60—100	20 ,,	224 ,,	5.	210—240	15 ,,	65 ,,
3.	120—170	20 ,,	137 ,,				

Fraction 1 was refractionated at atmospheric pressure, yielding (*a*) 28 g., b. p. 32—40°, $n_D^{20^\circ}$ 1·3940; (*b*) 2 g., b. p. 55—75°, $n_D^{20^\circ}$ 1·4290; (*c*) 4 g., b. p. above 75°, $n_D^{20^\circ}$ 1·4615.

20 G. of (*a*) (which was not further divisible by fractional distillation) were dissolved in cold dry carbon disulphide and treated with a 10% solution of bromine in the same solvent until the colour of the bromine just persisted after vigorous shaking and standing. After removal of carbon disulphide, the residue was distilled, and the fraction, b. p. 80—100°/20 mm., collected (yield, 35 g.). After several fractionations, a colourless liquid was obtained as the main portion, having b. p. 65—67°/13 mm., $n_D^{19^\circ}$ 1·5140, $d_{19^\circ}^{19^\circ}$ 1·6773, $[R_L]_D$ 41·29 (Found : Br, by the Carius method, 72·1. $C_5H_{10}Br_2$ requires Br, 69·6%; $[R_L]_D$ 40·84).

In order to satisfy ourselves that under precisely similar conditions isoprene yields the tetrabromo-derivative, we brominated this hydrocarbon. The product was a heavy, yellow oil, b. p. 152—156°/12 mm., $n_D^{20°}$ 1·5978, agreeing well with previously reported values for isoprene tetrabromide (Staudinger, *Helv. Chim. Acta*, 1922, **5**, 765, gives b. p. 153—155°/12 mm.). Since in the case under consideration a dibromide only was formed, it is obvious that the low-boiling distillate was either a single amylene or a mixture of isomerides.

Oxidation of the amylene. 9 G. were shaken with a slight excess of 2½% potassium permanganate solution (23 g. $KMnO_4$). After treatment with sodium bisulphite, the solution was filtered and fractionally distilled through a long column. The first 60 c.c. of the distillate were treated with *p*-nitrophenylhydrazine in hydrochloric acid and, after 12 hours, the precipitate produced was twice crystallised from dilute aqueous alcohol; yellow crystals of acetone-*p*-nitrophenylhydrazone (m. p. and "mixed" m. p. 146°) were thus obtained.

Fraction 3 (b. p. 120—170°/20 mm.). This was distilled at 15 mm. and separated into the following fractions: (A) b. p. 110—122°, 16 g.; (B) b. p. 122—134°, 29 g.; (C) b. p. 134—144°, 40 g.; b. p. 152—162°, 26 g.; (E) viscous residue.

(A) was apparently a mixture of mono- and sesqui-terpenes ($n_D^{18°}$ 1·4782, $d_{4°}^{18°}$ 0·8459). Cyclisation by boiling with 98% formic acid (30 g.) for 3 hours yielded an oil which was distilled at 25 mm. into two main fractions: (1) 5 g., b. p. 86—100°, $d_{21°}^{21°}$ 0·8326, $n_D^{21°}$ 1·4560, $[R_L]_D$ 45·17 (calc. for $C_{10}H_{16}|^{=}_{2}$, 45·2). (2) 8 g., b. p. 130—140°, $n_D^{20°}$ 1·4850.

(1) was a colourless mobile oil with an odour closely resembling that of pinene. (2) was added to sub-fraction B, which, however, further distillation indicated to be an inseparable mixture and was not further examined.

Sub-fraction C was distilled over sodium and yielded as major portion a faintly greenish-yellow oil, b. p. 129—135°/12 mm., $n_D^{21°}$ 1·4915, $d_{21°}^{21°}$ 0·8734, $d_{4°}^{21°}$ 0 8716, $[R_L]_D$ 67·7 (calc. for $C_{15}H_{24}|^{=}_{3}$, 67·8). These constants are identical with those of bisabolene (Table III) (Found: C, 87·9, 87·6; H, 11·6, 11·8. Calc. for $C_{15}H_{24}$: C, 88·2; H, 11·8%).

20 G. of this substance were cyclised by boiling with 98% formic acid (40 g.) for 3 hours, and the product was distilled at 21 mm. and separated into: (1) 2 g., b. p. 132—135°, $n_D^{20°}$ 1·4870; (2) 1 g., b. p. 137—145°, $n_D^{20°}$ 1·4912; (3) 14 g., b. p. 140—145°, $n_D^{20°}$ 1·4965, $d_{20°}^{20°}$ 0·8994, $[R_L]_D$ 66·28 (calc. for $C_{15}H_{24}|^{=}_{2}$, 66·1).

The main fraction would appear to be very similar to one of the hydrocarbons obtained by Ruzicka (*loc. cit.*) by treating nerolidol with formic acid, the constants for which were b. p. 114—116°/12 mm., $d_{4°}^{20°}$ 0·9069, $n_D^{20°}$ 1·4964, $[R_L]_D$ 65·82.

The whole of (3) was heated with finely-powdered sulphur (5 g.) at 200—250° until no more hydrogen sulphide or mercaptan was evolved (16 hours). The volatile products of the reaction (4 g.) were distilled in a vacuum from the large amount of resinous matter produced, repeatedly distilled over sodium until colourless, and treated with a concentrated hot alcoholic solution of picric acid. After 12 hours, the deposited red oil was redissolved in alcohol. In the solution, after several weeks, there suddenly appeared yellow laminæ of picric acid mixed with long orange needles, which were mechanically separated. These commenced to shrink at 95° and melted to a clear red liquid at 108—111° (cadalene picrate has m. p. 114—115°). Similar treatment of somewhat less highly fractionated sesquiterpene products gave, on two occasions, the same orange crystals, but sufficient pure material has not yet been collected for mixed melting-point determinations with cadalene picrate.

Sub-fraction D was a somewhat more viscous and more deeply coloured oil. The constants $n_D^{16^\circ}$ 1·4990 and $d_{16^\circ}^{16^\circ}$ 0·8934 and the impossibility of obtaining constant-boiling fractions on redistillation indicate that it is a mixture containing a large proportion of the next fraction.

Fraction 4 (b. p. 170—210°/15 mm.). 20 G., on fractional distillation at 9 mm., gave: (A) 8 g., b. p. 180—190°, $d_{19^\circ}^{19^\circ}$ 0·9073, $n_D^{19^\circ}$ 1·5082, $[R_L]_D$ 89·39 (Found: C, 87·8; H, 11·6); (B) 6 g., b. p. 190—200°, $d_{19^\circ}^{19^\circ}$ 0·9157, $n_D^{19^\circ}$ 1·5130, $[R_L]_D$ 88·88 (Found: C, 88·0; H, 11·7; *M*, cryoscopic in benzene, 259. Calc. for $C_{20}H_{32}$: C, 88·2; H, 11·8%; *M*, 272. $C_{20}H_{32}|_3^{=}$ and $C_{20}H_{32}|_4^{=}$ require $[R_L]_D$ 88·76 and 90·46, respectively).

Both (A) and (B) were greenish-brown, rather viscous, and almost odourless oils. The latter seems to be identical with the compound of molecular weight 303, obtained by Staudinger (*loc. cit.*) by the distillation of caoutchouc, and assumed by him to be $C_{25}H_{40}|_4^{=}$ (see Table IV).

Treatment of (B) with boiling formic acid for 3 hours gave an isomeride, b. p. 190—200°/9 mm., $d_{20^\circ}^{20^\circ}$ 0·9258, $n_D^{20^\circ}$ 1·5090, $[R_L]_D$ 87·72 (calc. for $C_{20}H_{32}|_2^{=}$, 87·1).

Fraction 5 was a dark-coloured, viscous oil which awaits detailed examination.

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