CCLXIV. THE HYDROCARBONS OF PIG LIVER.

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CHANNON AND MARRIAN [1926] examined the unsaponifiable fraction of pig liver primarily with a view to investigating the possible presence in liver of the unsaturated hydrocarbon squalene. Their results showed that, although squalene was not present, pig liver contained a highly unsaturated hydrocarbon of apparently very high molecular weight, and some evidence was obtained that this hydrocarbon was of terpenoid nature. The presence of the hydrocarbon was deduced from the ready preparation of an insoluble bromide and of a hydrochloride from fractions of the unsaponifiable matter into which the more unsaturated constituents had been concentrated. Analysis of the former compound showed that it was the bromide of an unsaturated hydrocarbon. The hydrochloride however was contaminated by an impurity which could not be removed and the mean values for the C, H and Cl analyses totalled no more than 98.71 %. The investigation of the unsaturated hydrocarbon did not progress further, because it could not be obtained in pure form; concentrates of it decomposed when distilled *in vacuo*, and regeneration by dehalogenation of its halogen compounds was unsatisfactory. Further, deductions as to the molecular formula of the substance from consideration of the analyses of the halogen compounds could not be made, for the molecular weight of the hydrocarbon was unknown, nor could those of the bromide and hydrochloride be determined on account of their properties. The great advances which have been made in knowledge of the chemical nature of the higher terpenes in the last few years and the increasing realisation of the biochemical importance of these substances suggested that further investigation of the liver compound was desirable. It seemed not inconceivable that this compound might be concerned with the metabolism of carotene and vitamin A, or possibly with that of cholesterol, for the feeding of squalene to rats causes a noteworthy increase in the amount of cholesterol in the liver [Channon, 1926]. This paper therefore contains an account of attempts to obtain the unsaturated hydrocarbon of mammalian liver in pure form as a preliminary to its chemical investigation. Sufficient progress has been made to lead us to publish the results so far obtained, for further advance cannot be made until more material is available, the preparation of which will entail a considerable delay.

EXPERIMENTAL.

Preparation of the unsaponifiable fraction.

Pig liver, having been shown to be richer in the hydrocarbon than that of the other mammals investigated, was again used as the starting material. The method adopted for the preparation of the unsaponifiable matter was essentially that used by Channon and Marrian [1926]. The pig liver was obtained from the

slaughter house and after removal as far as possible of the contaminating fat and larger vessels, the liver was minced into an equal volume of 5 % aqueous sodium hydroxide solution and the mixture heated for 6–7 hours on the water-bath with frequent shaking. In all cases the liver was in process of saponification within 6 hours after removal from the animals. One difficulty encountered in this procedure was that usually there were formed small coagulated lumps of unhydrolysed liver which made the extraction in the separators difficult. Decantation of the clear fluid from these lumps was in general unsatisfactory because of the bulk of the material. The difficulty was overcome finally by an initial incubation of the alkaline liver mixture at 37° for 12 hours. This treatment resulted in the formation of a uniform gel, further heating of which on the water-bath for a minimum of 8 hours gave a clear hydrolysate which could be readily extracted.

The solution was cooled, diluted with an equal volume of distilled water and poured into 15 l. aspirators. The early batches, which were not incubated, needed filtration through cheese cloth. Ether (3 l.) and alcohol (0.4 l.) were added while the liquid was still warm and the mixture shaken and allowed to stand overnight. This point is mentioned because it was found that these proportions and the method of addition to the slightly warm solution with the standing overnight caused the first extract to contain a very high proportion of the total ether-soluble material and, further, avoided all difficulties due to subsequent emulsification. Five to seven further extractions with ether alone were given, the procedure being to continue the extraction until the extracts were colourless. The combined ethereal extracts were washed with water until neutral to phenolphthalein.

After removal of the ether by distillation the residue was dried at 100° *in vacuo* with the addition of alcohol from time to time to assist the removal of residual water. The crude unsaponifiable matter so obtained was then resaponified by boiling for 2 hours with *N* sodium ethoxide. After boiling for one hour more, following the addition of a small quantity of water, as much alcohol as possible was removed by distillation. The residual mixture was then poured into water and extracted nine times with ether. The combined extracts were washed with water until neutral, the solvent removed and the residue dried at 100° *in vacuo* for from one-half to one hour. Such a drying procedure does not effect the removal of all solvent from a semi-solid material of this type, but it was decided to avoid submitting the material to further heating in order to minimise possible oxidative changes. An accurate final weight of the material was obtained and the first analyses were made at a later stage after partial fractionation had been carried out, for when the bulk of the cholesterol had been removed by crystallisation there remained an oil the accurate weight of which could be obtained readily after removal of the solvent by drying *in vacuo* on a water-bath.

It may be useful however to record the yields at this stage. 28.57, 47.88 and 2.1 kg. of liver yielded after the first saponification 169, 285 and 9 g. of crude unsaponifiable matter; these amounts correspond to 0.59, 0.59 and 0.43 % respectively. On resaponification the latter two samples were combined and yielded 233 g., or 0.46 %. Owing to a breakage the yield from the former sample, 169 g., cannot be recorded; the actual amount obtained however was 60 g. These two amounts were combined to give 293 g. of unsaponifiable matter, which was the starting material for the remainder of the work described. For the purposes of the subsequent discussion it is necessary to have some idea as to the amount of liver represented by this 293 g. This cannot be accurately given because of the loss already mentioned. However, since the yield on the first sample of liver, 28.57 kg., after the first saponification was identical with that of the second

sample, 47.88 kg., namely 0.59 %, it will serve the purpose if the assumption be made that the first sample would have yielded proportionately the same percentage on the second saponification as did the 47.88 kg. sample. If this be done, the starting material, 293 g., represents 62.9 kg. of liver.

In all further attempts at fractionating this material the effect of the successive processes in increasing the hydrocarbon concentration has been followed by routine determinations of iodine value and the yield of insoluble bromide. It will make the subsequent reading clearer if the method of calculation of the amount of hydrocarbon in a given fraction be described.

Insoluble bromide.

If a solution of the hydrocarbon be brominated in ether at a temperature of -15° to -20° , there is precipitated an ether-insoluble bromide, which may be filtered on a Gooch crucible, dried and weighed. It will be shown later in this paper that the hydrocarbon has one of the two formulae $C_{45}H_{76}$ or $C_{50}H_{84}$. The bromides of these compounds contain 67.5 and 67.7 % Br respectively and from the yield of insoluble bromide from any given fraction the amount of hydrocarbon has therefore been calculated as the mean value 32.4 %. This will only be an accurate procedure provided the yield of bromide is quantitative. Early in this investigation discordant results in estimations of the amount of hydrocarbon in a certain fraction led us to investigate the matter. Experiments in which different aliquots of the same fraction were brominated under identical conditions showed that the yield of insoluble bromide decreased markedly with increase in the volume of solvent used, even though the bromide when isolated had a solubility in ether at room temperature of only a few mg. per 100 ml. Thus in one experiment 17.1 mg. of a certain fraction when brominated in 5 ml. ether yielded 14.1 mg. of bromide; while 100.9 mg. dissolved in 4 ml. ether yielded 134.8 mg. of bromide. These two yields correspond to the fraction containing 26.6 and 43.3 % of hydrocarbon respectively. In other experiments in which the concentration of the same material was varied the yields of bromide corresponded to 7.6 and 9.8 % in one case and in another to 43 and 49 %. In the later work therefore we carried out the routine bromide estimations by the addition of bromine to a 2-3 % solution of the fractions in ether at -15° to -20° . We may say here that when the pure hydrocarbon was isolated as described later, it gave a quantitative yield when brominated under these conditions. The point arises however as to whether the yield of bromide is quantitative when a concentrate containing other substances is used. Our general impression, obtained after a very large number of routine determinations, leads us to believe that the presence of the other constituents has no marked influence on the bromide yield when carried out as described above.

Removal of cholesterol from the unsaponifiable matter.

The very great solubility of the non-cholesterol constituents of the unsaponifiable matter, together with the insolubility of cholesterol, make light petroleum, B.P. 40° - 60° , the most suitable solvent for the removal of the latter. Accordingly the unsaponifiable fraction, dissolved in warm light petroleum, 4 l., was crystallised at room temperature and filtered. Exhaustive subsequent fractionations of the residue and mother-liquor were then carried out in successively smaller volumes of light petroleum at 0° .

From the 293 g. of starting material there were obtained 160 g. of moderately pure cholesterol, together with 85.3 g. of a deep red oil, a total of 245 g. There was thus an apparent loss of 47.7 g. This apparent loss was not due to any significant extent to actual losses of material, because scrupulous care was taken to wash out every vessel with a suitable solvent. By far the greatest part of this loss therefore represents the amount of solvent in the original crude unsaponifiable matter present for the reason already mentioned.

Previous experience had shown that at this stage further cholesterol could be removed by the use of acetone. The system adopted therefore for the further fractionation was to crystallise the oil from acetone, and the precipitates so obtained were then recrystallised from light petroleum, which served to purify the cholesterol and to prevent loss of the non-sterol material. The temperatures used in the very tedious fractionation carried out varied between 0° and -17° . There were finally obtained 66 g. of oil of i.v. 208 and containing 12 % cholesterol, together with 12 g. of relatively pure cholesterol and an intermediate fraction, 5 g., which was not further investigated. Calculations may now be made as to the actual yields of cholesterol and the cholesterol-free oil; 63 kg. of liver yielded 180 g. cholesterol and 58 g. of oil, or 0.28 and 0.092 % respectively, a total yield of unsaponifiable matter of 0.37 % compared with the average figure of 0.36 % for four batches of $\frac{1}{2}$ cwt. each obtained by Channon and Marrian [1926].

Since the limits of removal of cholesterol by crystallisation had been reached, the remaining 8 g. of cholesterol present in the oil were removed by means of digitonin.

Removal of cholesterol by digitonin.

16.5 g. of the oil were dissolved in 600 ml. of 95 % alcohol, and the boiling solution was treated with 10 g. of digitonin dissolved in 700 ml. of boiling 90 % alcohol. The mixture was heated on the water-bath and after standing overnight was filtered and the residue washed with ether. As filtration was slow, a second washing of the residue was effected by transferring it to the flask in which the precipitation had been carried out and shaking with fresh ether. The total filtrate was then evaporated to dryness and an attempt made to extract the oil with light petroleum and to remove the excess of digitonin by filtration. Difficulty was again encountered in this procedure, and in order to minimise the exposure of the concentrate to the atmosphere the whole of the material was transferred to a large separating funnel by means of benzene and extracted several times with water at 60° with the addition of alcohol to effect separation. Evaporation of the washed benzene solution *in vacuo* yielded finally 14 g. cholesterol-free oil, fraction 1, i.v. 223. The weight of digitonide isolated (8.22 g.) corresponds to 2 g. cholesterol.

Treatment of the cholesterol-free oil with ether-methyl alcohol.

Channon and Marrian [1926] found that extraction of the oil with methyl alcohol removed a large proportion of the more saturated material. Owing to the insolubility of the hydrocarbon A¹ in methyl alcohol, it was desirable to seek improvement of this method. After a number of trials the procedure finally adopted was to dissolve the oil in a small volume of warm ether and to add

¹ The expression "hydrocarbon A" will be used to denote the unsaturated hydrocarbon referred to in the introduction. This hydrocarbon has an iodine value of 333 and later in this paper evidence is adduced which points to its formula being $C_{45}H_{76}$ or $C_{50}H_{84}$.

excess of warm methyl alcohol. This process was applied to 13.9 g. of fraction 1 in accordance with the scheme below.

Fig. 1.

By this treatment the material was resolved into two fractions, (2a) the more unsaturated fraction 8.95 g., i.v. 267, and (2b) the more saturated fraction, 4.96 g., i.v. 141.

The remainder of the material containing 12 % of cholesterol, 49.5 g., was therefore similarly treated both for the removal of cholesterol and the concentration of the hydrocarbon, during which some mechanical loss occurred. The corresponding fractions were united with those just described. The sterol-free fraction 1 was thus divided into fraction 2a, the material insoluble in ether-methyl alcohol, 32.0 g., i.v. 268, hydrocarbon A percentage 64.4 %, and fraction 2b, the material soluble in ether-methyl alcohol, weight 18.95 g., i.v. 140, hydrocarbon A percentage 4.1 %. Fraction 2a was then boiled with methyl alcohol, 200 ml., the mixture cooled at 0° and the clear mother-liquors decanted. This process was repeated three times and the mother-liquors were united. The insoluble fraction 3a (29.2 g., i.v. 258, hydrocarbon A 69.9 %) was dissolved in ether 50 ml. and, under reflux, methyl alcohol 100 ml. was added with constant shaking over a period of five minutes. The ether was removed by distillation and the mixture cooled in the ice-chest for several hours, when the mother-liquor was decanted. This process was repeated six times and yielded the insoluble fraction 4a, 25.45 g., i.v. 266, hydrocarbon A 72.1 %, and the soluble fraction 4b, 3.34 g., i.v. 182, hydrocarbon A percentage 29.9 %. A summary of the whole fractionation is recorded in Fig. 2.

It is of interest at this point to consider possible oxidative changes which may have taken place during the seven months occupied by the fractionation recorded in Fig. 2. Study of the composite iodine values at the different stages shows that surprisingly little, if any, oxidation had occurred. Thus the composite iodine values in stages 1, 2, 3 and 4 are 202, 218, 249 and 256, whereas the materials before each stage had an experimentally determined iodine value of 208, 223, 268 and 258 respectively. Although these differences may imply that a very slight degree of oxidation may have occurred, they are all save one within the range of experimental error. This finding as to stability has been borne out by all our subsequent experiences.

Summary of fractionation of the unsaponifiable matter.

Fig. 2.

The apparent amount of hydrocarbon present at each stage may also be calculated. The figures obtained are 18.3, 21.2, 20.6 and 19.2 g. respectively for stages 1 to 4. We believe the divergencies among these figures to be of no significance, because we had failed to appreciate at this stage the necessity of brominating under the conditions mentioned on p. 2014.

Distillation of fraction 4a.

Previous work had indicated that the hydrocarbon could not be distilled in a vacuum attainable with the usual laboratory apparatus and, as distillation seemed the most promising method of purification, recourse was had to high vacuum distillation. In this we were fortunate in having the generous help of Dr F. H. Carr, who submitted 9.9 g. of the material to this treatment. The

material was distilled in four portions and no obvious sign of decomposition occurred. The distillates were collected into three fractions, 5a, 5b and 5c, details of which, together with their hydrocarbon A percentages and iodine values, are given in Table I.

Table I. *Distillation of 9.9 g. of fraction 4a at 10⁻⁵ mm.*

Fraction No.	(g.)	Temperature	I.V.	Hydrocarbon %
5a	1.45	160°-285°	97	9.9
5b	5.25	310°-370°	(3) 329	49.2
5c	R 0.9	Above 370°	247	0.0
	7.6			

Composite I.V. 275. Original I.V. 266.

It is to be observed that the distilled material and residue together amount to 7.6 g. out of the original 9.9 g. of starting material. The difference is entirely due to mechanical loss. Further, there has been an obvious separation of more saturated constituents into fraction 5a, with a concentration of the more unsaturated material into fraction 5b. The undistilled residue of fraction 5c shows a fall in iodine value compared with fraction 5b, which is to be expected. At first sight these results would suggest that fraction 5b consisted of pure hydrocarbon, for its iodine value, 329, is approximately that required by hydrocarbon A. When we came to investigate this by the yield of insoluble bromide produced we found, however, that it contained no more than 49.2 % or 2.58 g. This suggests that during distillation some change, possibly isomerisation of the material, had occurred in part. Bromination experiments carried out on fraction 5a showed 9.9 % of hydrocarbon A to be present, or 0.14 g.; fraction 5c gave no insoluble bromide. Hence the determinations of bromide yield suggest that the three fractions contain together 2.72 g. of hydrocarbon, whereas the original starting material, 9.9 g., contained 7.14 g. There is thus an apparent loss of 62 % of the hydrocarbon. It has to be remembered however that the material returned from the distillation was 2.3 g. less in weight than that which was provided. This mechanical loss is reported to have occurred in each fraction in proportion to the size of the fraction. However, even if all the missing material were pure hydrocarbon A, the total hydrocarbon would only be 5.02 g. or about 70 % of that originally present. It is clear from these results therefore that, although the hydrocarbon can be distilled in high vacuum of this type, it undergoes to a serious extent some change which causes it no longer to yield insoluble bromide. In view of these results we decided to attempt purification by adsorption methods.

In order to gain some insight into the applicability of such methods to the present problem, a preliminary experiment was carried out with the unsaponifiable matter of the liver oil of *Scymnorhinus lichia*. This contains a large proportion of squalene, of which hydrocarbon A is possibly a homologue, and in addition cholesterol and other higher alcohols. By saponification of 30 g. of liver oil with alcoholic potassium hydroxide there were obtained 13.56 g. unsaponifiable matter of iodine value 242. 10 g. of this oil dissolved in 500 ml. light petroleum were filtered slowly through a column of aluminium oxide measuring 70 × 1.8 cm. diameter, which was washed with further quantities of 200 ml. solvent. Each of the filtrates was evaporated separately to dryness and its weight and iodine value determined. The primary filtrate and first washing yielded 4.103 g. and 1.632 g. respectively of a mobile colourless oil, of iodine value 364, consisting

evidently of almost pure squalene, which has i.v. 371. The second and third washings contained only 22 mg. (i.v. 280) and 6 mg. (i.v. 244) of oil respectively. The column was divided into three equal portions which were eluted with dry ether. From the top section 2.0 g. of a white wax were obtained and from the middle section 0.4 g. of a semi-liquid wax. The adsorption which had occurred in the lower section was negligible as only 3 mg. of material were obtained.

From the iodine value of the original material, 242, the maximum squalene percentage, even assuming no other unsaturated constituent to be present, was 65.2 %, while the primary filtrate and first washing yielded 57.4 % of virtually pure hydrocarbon. Bearing in mind that other unsaturated alcohols are present in the unsaponifiable matter of the livers of these sharks, it is clear that the recovery of squalene is virtually quantitative. As hydrocarbon A is probably terpenoid in character [Channon and Marrian, 1926] it seemed that a similarly efficient separation might be obtained in the present case, save in so far as it might be modified by the more complex nature of the hydrocarbon.

Fractionation of the concentrate fraction 4a by adsorption on aluminium oxide¹.

12.797 g. dissolved in light petroleum (1.1 l.) were filtered through a column of aluminium oxide, 70 cm. in height and 1.8 cm. in diameter, in 6½ hours. 80 ml. light petroleum were used to wash the column and the combined filtrates were evaporated to dryness. This primary filtrate yielded 1.301 g. of a colourless mobile oil, fraction 7a. Further washing of the column with 300 ml. of light petroleum yielded fraction 7b, 0.078 g. of material of the same appearance as fraction 5. A third washing with 800 ml. of solvent yielded 0.057 g., fraction 7c. The filtrate from a further treatment with 800 ml. of solvent yielded 0.503 g., fraction 7d. A study of the amounts of material enabled some deductions to be made as to the effect of the different washings. The first filtrate, together with the first two washings, yielded a colourless oil in decreasing amounts, the second washing, 800 ml., having yielded in fraction 7a no more than 57.2 mg. The third washing with 800 ml., however, yielded an amount of material which, in further contrast, was a viscous yellow oil and which was some ten times greater than fraction 7c. These considerations pointed to fractions 7a, b and c being composed of one or more substances and to fraction 7d being of a markedly different chemical character. During these washings with 1.9 l. of solvent there was no obvious separation of the column into layers of different colour. The colour progressively decreased from buff through graded shades to pale cream and to white at the lower end. The column was therefore removed and cut into four sections, the upper 13 cm., which were buff-coloured, and the lower 26 cm., which were white, while the middle 31 cm. were divided into two equal portions. The various sections were then eluted by extraction with boiling benzene-alcohol 10:1 five times. Each extract was evaporated to dryness and then dissolved in light petroleum and filtered to remove final traces of finely divided alumina. The solutions were then evaporated, dried at 100° *in vacuo* and weighed. The total yield of material was 12.374 g. or 97 %. The iodine values of each of the fractions, other than the small amounts of fractions 7b and 7c, were determined. Details of this analysis are recorded in Table II.

It is clear from the data recorded in Table II that a marked separation of the components of the original fraction 4a has been effected.

¹ This material, Aluminiumoxyd für Adsorptionszwecke nach Brockmann, was obtained from E. Merck, Darmstadt.

Table II. *Adsorption analysis of fraction 4a.*

Fraction No.	Source	Wt. g.	Description	I.V.
7e	Eluted from top quarter	2.737	Red brown very viscous oil	239
7f	„ 2nd „	2.574	—	320
7g	„ 3rd „	2.544	—	330
7h	„ 4th „	2.579	Pale yellow oil	333
7a	Primary filtrate	1.301	Colourless oil	17
7b	First washing	0.078	„	—
7c	Second washing	0.057	„	—
7d	Third washing	0.503	—	123
Total		12.373	(recovery 97 %)	

Fraction 7a. From the iodine value of 17, this fraction clearly consists of a saturated substance contaminated by a relatively small proportion of unsaturated material. We endeavoured to remove the latter by passing 1.29 g. of fraction 7a in solution in light petroleum, 100 ml., through a fresh column. The process was unsuccessful, for the iodine value of the product obtained, 1.09 g., was still 13.6. On analysis fraction 7a gave C 85.89, H 13.71, while its molecular weight in camphor was 417. This substance is therefore a saturated hydrocarbon, which will be designated as hydrocarbon B in the discussion which follows. The most likely explanation of the iodine value is that a small amount of hydrocarbon A was present in it, for the latter constituted a considerable proportion of fraction 7d, the third washing, and it would need only 5 % of hydrocarbon A to give the mixture an iodine value of 16. An attempt was made to prove this by bromination of 101.1 mg. in 1 ml. ether at -18° . There was no insoluble bromide formed. Since the presence of 5 % hydrocarbon A would correspond to 15 mg. bromide, which are capable of quantitative treatment, it is evident that the unsaturated nature of the mixture is due to an unsaturated hydrocarbon other than A. This point is referred to again in the later analysis of fraction 10.

Hydrocarbon B was also obtained by the adsorption technique from fraction 5a; this first distillate, 0.568 g., i.v. 97, yielded 0.319 g. of i.v. 11, from which it is clear that it may be separated from hydrocarbon A by fractional distillation. No analyses were made on fractions 7b and 7c, but if these be included with fraction 7a, a total of 1.436 g. or 11.2 % of almost saturated material has been removed from a material having an original iodine value of 266.

Fraction 7d. The change in appearance of fraction 7d from that of the preceding washings, together with its iodine value, made it seem probable that it was largely composed of hydrocarbon A and it was accordingly brominated. 76 mg. yielded 42.6 mg. of bromide, which corresponds to 18.2 % of hydrocarbon A. That this bromide was that of hydrocarbon A was confirmed by analysis (C 28.50, H 3.97, Br 67.09 %). This percentage only accounts for the fraction having an iodine value of 60 as against the 123 found. Calculation shows that that part of the fraction which was not hydrocarbon A would thus have an iodine value of 77, a fact which will be referred to again.

Fractions 7e-7h. Study of the iodine values in Table II shows that there is a progressive separation of the more unsaturated material with increasing depth of the column. The percentages of hydrocarbon present in fractions 7e-7h determined as bromide were 65.8, 78.4, 91.6 and 100 % respectively. Although the latter figure accounts fully for the observed iodine value of fraction 7b, in the other cases the found hydrocarbon percentage fails to do so. Calculation shows that the material in fractions 7e, 7f, 7g, which is not hydrocarbon A, has iodine

values of 58, 273 and 298 respectively. Two things are apparent from these figures. Either the estimation of the hydrocarbon in these fractions is not quantitative, or else they contain another highly unsaturated compound. Since the total yield of hydrocarbon from all the fractions 7*a*–7*h* is 8.9 g., or 71.9 % of the starting material, on which the found percentage was 72.1 %, the latter seems the more probable alternative.

Fraction 7*e* clearly contains a saturated substance in addition and this substance contains a considerable proportion of oxygen, for whereas fractions 7*f*–7*h* gave combustion analyses approaching 100 %, the figures for 7*e* were C 81.1, H 11.2 %.

Fraction 7h. This fraction is the pure hydrocarbon A, on which the following analyses were carried out:

(*a*) 69.8 mg. gave 216 mg. bromide, which gave on analysis C 28.24, H 3.96, Br 67.35 %. This yield of bromide is 100 % of the theory on a basis of the bromine percentage of 67.35 %. The bromide on heating changed in colour from white to purple at 125°; it turned from brown to black between 165 and 180° and did not melt.

(*b*) Hydrogen chloride was passed into a solution of 215.8 mg. in acetone 8 ml. at –10°. After filtration and washing with four portions of 2 ml. ether during which a slight loss occurred, the yield of hydrochloride obtained, 276.0 mg., was 89.7 % of the theoretical, even when the correction for its solubility in acetone is neglected. The hydrochloride so obtained was not quite white; it was therefore treated with charcoal in acetone solution and finally obtained as a white solid, containing C 59.38, H 9.07, Cl 31.25 %. This melted indefinitely between 115 and 121°, and its behaviour during melting was such as to suggest that it may have been a mixture of isomerides (*cf.* squalene). The amount of material was insufficient to determine this, but it may be mentioned that in one preparation, from fraction 5*b*, a melting-point of 132° was obtained. Channon and Marrian [1926] record 128° as the melting-point of their somewhat impure preparation.

(*c*) Combustion analysis gave C 87.23, H 12.30 % and two independent molecular weight determinations in camphor gave 609, 618.

The very high molecular weight of the hydrocarbon and the analytical data on its halogen derivatives make accurate deductions as to the empirical formula somewhat uncertain. The ratios of carbon to hydrogen in the analyses of the hydrocarbon, its bromide and hydrochloride, are 1.69, 1.68 and 1.66 respectively, and since experience has shown with other compounds which are oils of high molecular weight that determinations of the latter may yield results which may be no more than figures of the correct order, we are reluctant to express any definite opinion as to the formula of the compound. However, if the evidence previously given that the compound is a terpene be confirmed, and if the frequency with which multiples of isoprene units appear in natural products be accepted as making such a multiple a reasonable presumption for this substance, calculations may be made as to the possible formulae. For this purpose there are recorded in Table III the theoretical figures for three such compounds and their halogen derivatives, together with those of squalene and those experimentally found for hydrocarbon A.

The analyses of these compounds provide evidence which on the whole favours $C_{45}H_{76}$ as being a more probable formula than $C_{50}H_{84}$, but it has to be recognised that the limits of analysis have been virtually reached. In favour of the former formula is the fact that the molecular weight required is 617 as against the 613 found. For reasons already stated we do not consider this a significant

Table III.

	Double bonds	Hydrocarbon			Bromide				Hydrochloride			
		Mol. wt.	C	H	Mol. wt.	C	H	Br	Mol. wt.	C	H	Cl
$C_{40}H_{68}$	7	549	87.5	12.5	1667	28.8	4.1	67.1	804	59.7	9.40	30.9
$C_{45}H_{76}$	8	617	87.6	12.4	1895	28.5	4.0	67.5	909	59.5	9.3	31.2
$C_{50}H_{84}$	9	685	87.6	12.4	2123	28.3	4.0	67.7	1013	59.3	9.3	31.5
Found		613	87.2	12.3	—	28.2	4.0	67.35	—	59.38	9.07	31.25
$C_{30}H_{50}$ (squalene)	6	410	87.8	12.2	1370	26.3	3.65	70.1	629	57.2	8.9	33.9

point, even though the $C_{50}H_{84}$ compound would require a molecular weight of 684.

Any compound which contained double bonds in a ratio of one double bond to 5.6 carbon atoms would yield halogen derivatives which would accord with the analyses found; but it seems improbable that compounds on the lower side of C_{45} need be considered, because of the behaviour on distillation. Squalene, $C_{30}H_{50}$, readily distils at 240° at 2 mm. pressure. Hydrocarbon A concentrates completely decomposed under these conditions and even in the distillation carried out by Dr Carr at 10^{-5} mm. the temperature range over which fraction 5b distilled was $310\text{--}370^\circ$, and then much of the hydrocarbon had undergone some change which made it no longer yield an insoluble bromide. This implies a considerably higher molecular weight than that of squalene, and the only compound below C_{45} which might be considered is $C_{40}H_{68}$. It seems reasonable however to doubt whether the increase in the carbon chain by ten carbon atoms would have this very profound effect on the ease of distillation. These reasons and the analytical results suggest that the compound is probably $C_{45}H_{76}$ or possibly $C_{50}H_{84}$. The iodine values required by these two compounds are 329 and 333 respectively. The found value was 333. One other point needs mention. The next higher or lower homologue of either of these compounds is ruled out by the analytical results; for example $C_{40}H_{68}$ would require for its bromide C 27.9, H 3.9, Br 68.2 % and for its hydrochloride C 58.9, H 9.2, Cl 31.95 %.

The fraction soluble in methyl alcohol, fraction 5.

The many treatments of the original cholesterol-free material with ether-methyl alcohol had resulted in the fraction soluble in methyl alcohol containing some of the unsaturated hydrocarbon A and it was decided therefore to attempt to remove this by treatment with aluminium oxide. The procedure was similar to that used for the insoluble fraction, save that in the first treatment the material was passed through a column in approximately 2 % solution.

21.34 g. of material of iodine value 140 were dissolved in light petroleum 1 l. and the solution was filtered through a column 66 cm. long and 1.8 cm. in diameter. The colour diffused rapidly down the column and had reached the bottom after the passage of 850 ml. of the solution. By far the greater part of the filtrate was colourless, but the last 100 ml. together with 60 ml. of washing resulted in the material obtained from this primary filtrate being yellow. The column was then washed with successive volumes of light petroleum of 300 ml., 300 ml. and 350 ml. respectively and the material in each of these washings was obtained by evaporation to dryness *in vacuo*. The progressively decreasing weights of these washings (fractions 8b to 8d of Table IV) made it appear that separation by this means had virtually come to an end with the third washing. The column, the colour of which was graded from cream at the top to buff at the bottom, was divided into three portions and the material eluted from the alumina by six

treatments with a boiling mixture of benzene-alcohol 10 : 1. The weights of the fractions, together with the iodine values and descriptions of the material obtained, are recorded in Table IV.

Table IV. *Adsorption analysis of the fraction soluble in ether-methyl alcohol, F 5.*

Fraction No.	Source (cm. from top of column)	Wt. g.	I.V.	Description
8e	0-10	0.820	88.6	Orange waxy solid at room temp.: red oil when molten
8f	11-21	3.737	126.0	As 8e
8g	22-62	5.601	162.0	Red viscous oil, just flowing at room temp.
8a	Primary filtrate	5.317	172.0	Red-brown mobile oil
8b	First washing	1.730	156.0	Red-brown oil, more viscous than 8a
8c	Second washing	0.560	159.0	Red-brown oil, paler than 8b
8d	Third washing	0.134	—	Golden yellow oil
		17.899 g. from 21.34 g.		

It will be observed that the recovery of material represents no more than about 84 % and that the iodine values of the different fractions imply that no substantial separation of the constituents has been effected.

Further treatment of fraction 8a, the primary filtrate.

5.3 g. of this fraction, which should have contained any of the saturated hydrocarbon B, were dissolved in 500 ml. of light petroleum and passed through a column 42 cm. long and 1.2 cm. in diameter. All the colour was adsorbed in the top quarter of the column. After filtration the column was washed with a further 750 ml. of light petroleum, and even at the end of this washing the bottom third of the column remained quite white. Evaporation of the solution and filtrate to dryness yielded fraction 9a, 2.559 g. of a colourless mobile oil, insoluble in hot and cold ethyl alcohol and having an iodine value of 73, as against that of 172 of the original fraction 8a. This big decrease in iodine value suggested a further treatment with alumina, and accordingly 2.5 g dissolved in 250 ml. of light petroleum were passed through a column 31 cm. long and 1.2 cm. in diameter. The column in this case was washed with 20 ml. of light petroleum only. The combined filtrate and washing yielded on evaporation fraction 10, 2.443 g. of oil of iodine value 72. This passage of fraction 9a through the column thus resulted in the adsorption of only 2 % of the material and the iodine value was consequently unchanged. The limits of treatment by this method having been reached, an analysis of this product gave C 86.27, H 13.33, mol. wt. (camphor) 314, 317. These figures show clearly that this material is hydrocarbon in nature, and the question arose as to whether the iodine value was not due to its being a mixture of the saturated hydrocarbon B and the unsaturated hydrocarbon A in proportions of about 3 : 1. In order to test this possibility a portion was brominated in ether. 97.9 mg. yielded 15.2 mg. of insoluble bromide, which behaved on heating like that of hydrocarbon A. This corresponds to the material containing 5.03 % of the unsaturated hydrocarbon A. Such a percentage would confer on this fraction an iodine value of 17 only, and calculation shows that the remaining 94.97 % must have an iodine value of 58. If this were due to a hydrocarbon containing one double bond reacting with iodine, its molecular weight would be 438. Since this material must almost certainly have contained some of the saturated hydrocarbon B, however, it appears probable that it is a mixture of the latter with an unsaturated hydrocarbon other than hydrocarbon A. The possibility that it

consisted of a mixture of hydrocarbons A and B alone is ruled out by the evidence above and also as the result of the following experiment.

It has already been noted that bromination of 101.1 mg. of hydrocarbon B (I.V. 14) failed to give any precipitate. In order to conserve material the bromine and solvent were removed at 50° *in vacuo*, 33.8 mg. hydrocarbon A added and the mixture dissolved in 2.5 ml. ether. Bromination at -20° gave 104.5 mg. bromide corresponding exactly to the amount of A added. The nature of this mixture of A and B does not therefore affect the quantitative precipitation of the bromide. It will be remembered that bromination of the hydrocarbon fraction 7*d* revealed the possibility of there being another hydrocarbon present, now designated as hydrocarbon C, of approximate iodine value 77 and somewhat more strongly adsorbed on the alumina than B. It is probable therefore that fraction 10 consists of a mixture of these two latter substances in the proportion of 1 : 3.

Ultra-violet absorption spectra.

As hydrocarbon A was pure and the further removal of impurities from B and C was not immediately possible by the methods at our disposal, the three substances were submitted to Dr S. R. Edisbury in 1 % hexane solution for determination of their absorption spectra in the ultra-violet region. Each of the hydrocarbons exhibited only weak general absorption. In addition hydrocarbons B and C showed an ill-defined range of selective absorption of very low intensity at 250–280 $m\mu$, probably due to the presence of traces of impurity of an aromatic nature, the absorption bands of which are superimposed on the general absorption of the hydrocarbons themselves.

As hydrocarbon A, containing 8 or 9 ethenoid linkages, does not show selective absorption, it is extremely improbable that as many as three double bonds are conjugated. An attempt to cyclise the hydrocarbon by treatment with alcoholic hydrogen chloride brought about a slight general fall in absorption intensity and the appearance of a shallow band at 286 $m\mu$. This suggests that a rearrangement of the unsaturated system has taken place to some slight extent, giving rise to a small quantity of material with three or possibly four conjugated linkages. It is improbable that appreciable cyclisation occurred unless any ring systems so formed were fully or nearly saturated.

SUMMARY.

1. The unsaponifiable matter from 63 kg. of pig liver has been prepared, the cholesterol quantitatively removed, and the sterol-free oil obtained (52 g.) has been investigated.

2. By exhaustive treatment with ether-methyl alcohol it was divided into an insoluble fraction, 25.5 g., of iodine value 266, and a soluble fraction, 24.8 g., of iodine value 140.

3. The insoluble fraction consisted to the extent of 70 % of the unsaturated hydrocarbon of iodine value 333, which was shown to be present by Channon and Marrian [1926]. The bromide and hydrochloride of this hydrocarbon have now been prepared in pure form and analysed. By an adsorption technique the hydrocarbon has been isolated in pure form. The analytical data suggest the formula $C_{45}H_{76}$ or $C_{50}H_{84}$. Attempts to distil the compound at 10^{-5} mm. resulted in marked change.

4. About 10 % of the insoluble fraction consists of a saturated hydrocarbon which has not yet been obtained pure. A further 10 % consists of a compound containing oxygen.

5. Further, it is shown that the methyl alcohol-soluble fraction contains a third hydrocarbon which contains one double bond reacting with iodine.

6. The yield of the unsaturated hydrocarbon $C_{45}H_{76}$ or $C_{50}H_{84}$ was 29 mg. per 100 g. of liver.

We wish to record our thanks to Dr F. H. Carr for kindly distilling one of the fractions in a high vacuum apparatus and to Dr R. A. Morton and Dr J. R. Edisbury for the spectrographic investigation of a number of fractions.

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