THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL.
VIII. THE CONSTITUTION OF CLUPANODONIC ACID $C_{22}H_{34}O_2$.

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As described in the preceding paper, we have separated from sardine oil a mixture of highly unsaturated C_{22}-acids which was resolved further into the portions of different degrees of unsaturation in order to obtain clupanodonic acid $C_{22}H_{34}O_2$ in a much purer state than prepared heretofore and also a more highly unsaturated fraction consisting chiefly of docosahexenoic acid $C_{22}H_{32}O_2$. The present paper deals with the constitution of clupanodonic acid. The same subject has hitherto been studied by Tsujimoto⁽¹⁾ and also by Inoue and Sahashi.⁽²⁾ On subjecting clupanodonic acid and its amyl ester to ozonolysis, Tsujimoto found propyl aldehyde, acetaldehyde, carbon dioxide together with comparatively large quantities of succinic acid, and also amyl hydrogen succinate in the case of amyl clupanodonate. Since acetaldehyde and carbon dioxide were considered to be formed by a further degradation of malonic aldehyde, the above results indicated that clupanodonic acid had the following groups: $CH_3{\cdot}CH_2{\cdot}CH{=}$, $={CH}{\cdot}CH_2{\cdot}CH{=}$, $={CH}{\cdot}(CH_2)_2{\cdot}CH{=}$ and $={CH}{\cdot}(CH_2)_2{\cdot}COOH$, of which it had three of the group $={CH}{\cdot}(CH_2)_2{\cdot}CH{=}$. In regard to the respective positions of the groups $={CH}{\cdot}CH_2{\cdot}CH{=}$ and $={CH}{\cdot}(CH_2)_2{\cdot}CH{=}$, he showed no experimental evidence, but he assumed clupanodonic acid to have one of the ethylenic linkings in the same position as cetoleic acid ($\Delta^{11:12}$-docosenoic acid) which is found to occur widely in marine animal oils, and ascribed to clupanodonic acid the following alternative formulae:

$CH_3{\cdot}CH_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}CH{=}CH{\cdot}CH_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}COOH$
or
$CH_3{\cdot}CH_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}CH{=}CH{\cdot}CH_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}COOH$.

By oxidation of clupanodonic acid with potassium permanganate in acetone, Inoue and Sahashi obtained butyric, pimelic, succinic, adipic,

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(1) This Bulletin, **3** (1928), 299.

(2) *Proc. Imp. Acad. Japan,* **8** (1932), 371.

malonic and oxalic acids, of which adipic and oxalic acids were assumed to have been formed by the secondary decomposition of pimelic and malonic acids respectively, and taking the yields of these oxidation products into consideration, they concluded that clupanodonic acid yielded butyric, pimelic, succinic (2 mols) and malonic acids as scission products derived from the respective groups jointed by the unsaturated linkings. In order to determine in what order the respective groups are jointed, these authors subjected clupanodonic acid to a partial hydrogenation, separated the hydrogenation products into the fractions of different degrees of saturation and examined each fraction by the permanganate oxidation with the results given in Table 1.

Table 1.

Clupanodonic acid and its partially hydrogenated products	Oxidation products (acids)				
Clupanodonic acid	Butyric, (C_4)	Pimelic, (C_7)	Succinic, (C_4)	Malonic, (C_3)	Succinic (C_4)
Partially hydrogenated products					
Fraction which yields a hexabromide	Undecanoic, (C_{11})		Succinic, (C_4)	Malonic, (C_3)	Succinic (C_4)
Fraction which yields a tetrabromide	Pentadecanoic, (C_{15})			Malonic, (C_3)	Succinic (C_4)
Fraction which yields a dibromide	Stearic, (C_{18})				Succinic (C_4)

Based on these results Inoue and Sahashi ascribed to clupanodonic acid the following formula having one triple and three double linkings:

$$CH_3\cdot(CH_2)_2\cdot C\equiv C\cdot(CH_2)_5\cdot CH=CH\cdot(CH_2)_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot(CH_2)_2\cdot COOH.$$

It is to be noted that the samples of clupanodonic acid used in the experiments of Tsujimoto and the subsequent authors were prepared by means of the lithium-soap-acetone method and the fractional distillation of methyl esters without applying a further separative operation, consequently they must have been contaminated with some other acids of different degrees of unsaturation as pointed out in our previous report.

In this paper, the same sample of clupanodonic acid as described in the preceding paper was converted into the amyl ester, and the latter was subjected to ozonolysis. Among the products of ozonolysis, propyl aldehyde, acetaldehyde, carbon dioxide, succinic acid and amyl hydrogen succinate were identified; the presence of some lower acids consisting possibly of propionic and acetic acids was also indicated. Of these compounds, propyl aldehyde is evidently derived from the group $CH_3\cdot CH_2\cdot CH=$, whilst acetaldehyde and carbon dioxide are attributable to the secondary decomposition of the product of ozonolysis derived from the group $=CH\cdot CH_2\cdot CH=$. Succinic acid and amyl hydrogen succinate are derived from the groups $=CH\cdot(CH_2)_2\cdot CH=$ and $=CH\cdot(CH_2)_2\cdot COOC_5H_{11}$ respectively. Accordingly clupanodonic acid has the following groups: $CH_3\cdot CH_2\cdot CH=$, $=CH\cdot(CH_2)_2\cdot COOH$, $=CH\cdot(CH_2)_2\cdot CH=$ and $=CH\cdot CH_2\cdot CH=$, of which it has three of the group $CH\cdot(CH_2)_2\cdot CH=$, since it contains 22 carbon atoms in its molecule. These results, however, give no information regarding the respective positions of the groups $=CH\cdot(CH_2)_2\cdot CH=$ and $=CH\cdot CH_2\cdot CH=$, and it requires a further examination to establish the constitution of clupanodonic acid. In the case of moroctic acid ($\varDelta^{4:5,\,8:9,\,12:13,\,15:16}$-octadecatetraenoic acid), we have determined the respective positions of the groups by preparing tetrathiocyanate (4,5,8,9-tetrathiocyanate) by the selective addition of thiocyanogen to methyl moroctate, and by examining this tetrathiocyanate by ozonolysis. We have, however, found by subsequent studies⁽³⁾ on the partial bromination of the poly-ethylenic acids of known constitution, such as linoleic and linolenic acids, that the addition of bromine took place selectively at the specified ethylenic linkings of these poly-ethylenic acids; though such a selective addition did not proceed to a quantitative extent, the products of stepwise bromination contained in every case a definite bromide formed by the selective addition as the main constituent which could be separated by means of a suitable separative operation. After having found the above fact, we have applied this to clupanodonic acid, and determined the positions of the groups $=CH\cdot(CH_2)_2\cdot CH=$ and $=CH\cdot CH_2\cdot CH=$ by examining the products of partial bromination of clupanodonic acid by ozonolysis. The results are summarized below. It deserves a special attention that whilst thiocyanogen added selectively to the two ethylenic linkings which existed near the carboxyl group of moroctic acid, bromine attacked preferentially the ethylenic linkings which were further removed from the carboxyl group of clupanodonic acid.⁽⁴⁾

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(3) *J. Soc. Chem. Ind., Japan,* **38** (1935), 89.

(4) The same relation was observed in the case of linoleic and linolenic acids, viz., whilst thiocyanogen attacked selectively the 9:10-ethylenic linking of linoleic acid and also the 9:10- and the 12:13-ethylenic linkings of linolenic acid, bromine attacked preferentially the ethylenic linking which was the further removed from the carboxyl group. Cf. Toyama and Tsuchiya, *J. Soc. Chem. Ind., Japan,* **38** (1935), 86, 89.

(1) Dibromo-derivative $C_{22}H_{34}O_2Br_2$ obtained by the addition of 1 mol bromine to 1 mol clupanodonic acid yielded, as the products of ozonolysis, acetaldehyde, carbon dioxide, succinic acid and an oily bromo-compound which on debromination and subsequent hydrogenation gave heptoic acid, indicating that the oily bromo-compound obtained above consisted of dibromoheptoic acid. Since clupanodonic acid was shown to contain the group $CH_3 \cdot CH_2 \cdot CH=$ by the ozonolysis of amyl clupanodonate, it follows from the above results that it contains the group $CH_3 \cdot CH_2 \cdot CH=CH \cdot (CH_2)_2 \cdot CH=$.

(2) Tetrabromo-derivative $C_{22}H_{34}O_2Br_4$ obtained by the addition of 2 mols bromine to 1 mol clupanodonic acid yielded, as the products of ozonolysis, succinic acid and an oily bromo-compound which on debromination and subsequent hydrogenation gave capric acid. This fact, coupled with the result obtained by the ozonolysis of dibromo-derivative, indicated the presence of the group $CH_3 \cdot CH_2 \cdot CH=CH \cdot (CH_2)_2 \cdot CH=CH \cdot CH_2\ CH=$.

(3) Hexabromo-derivative $C_{22}H_{34}O_2Br_6$ was prepared by the addition of 3 mols bromine to 1 mol clupanodonic acid. Ozonolysis of this bromide yielded succinic acid and an oily bromo-compound, from which myristic acid was obtained by debromination followed by hydrogenation. Hence clupanodonic acid was found to contain the group
$CH_3 \cdot CH_2 \cdot CH=CH \cdot (CH_2)_2 \cdot CH=CH \cdot CH_2 \cdot CH=CH \cdot (CH_2)_2 \cdot CH=$.

From these results the constitution of clupanodonic acid was established as follows:

$$CH_3 \cdot CH_2 \cdot CH=CH \cdot (CH_2)_2 \cdot CH=CH \cdot CH_2 \cdot CH=CH \cdot (CH_2)_2 \cdot CH=CH \cdot (CH_2)_2 \cdot CH=CH \cdot (CH_2)_2 \cdot COOH.$$

Comparing the results of our experiments with those obtained by Tsujimoto, they show a full agreement in that clupanodonic acid consists of the groups: $CH_3 \cdot CH_2 \cdot CH=$, $=CH \cdot (CH_2)_2 \cdot CH=$ (three), $=CH \cdot CH_2 \cdot CH=$, and $=CH \cdot (CH_2)_2 \cdot COOH$. While Tsujimoto derived two of his formulæ by assuming that clupanodonic acid had one of its ethylenic linkings in the same position as that of cetoleic acid ($\Delta^{11:12}$-dococenoic acid), the result of our further research in order to determine the positions of the respective groups, however, indicated that the above assumption of Tsujimoto was not substantiated, since clupanodonic acid, unlike cetoleic acid, had no ethylenic linking in the 11:12-position. Consequently Tsujimoto's formula should be corrected. Comparing the formula given by Inoue and Sahashi with that established by us, they show much difference with the exception that both formulæ contain the group $=CH \cdot (CH_2)_2 \cdot COOH$ at the side of the carboxyl group. Particularly, the former is incompatible with the latter in that it contains a C_4-group at the side of the methyl group and a C_7-group in the adjascent place, having a triple linking between these two groups. The reason why these discrepancies occur is unknown to us. According to the results given by these authors, not only clupanodonic acid itself but also its hydrogenated products having one to three ethylenic linkings yielded on oxidation only the compounds corresponding just to the formula given by these authors, but it is quite difficult to understand the formation of the oxidation products found by these authors, such as undecanoic, pentadecanoic and stearic acids with the use of our formula, unless displacements of the unsaturated linkings occur in the course of hydrogenation. Although the possibility is not excluded that the permanganate oxidation method, when applied to a highly unsaturated acid like clupanodonic acid, gives rise to abnormal oxidation products which differ in the number of carbon atoms from those obtained by the ozonide method, it is rather inconceivable that such abnormal decomposition takes place also in the hydrogenated products of clupanodonic acid having one to three unsaturated linkings when the permanganate oxidation method is applied. For it has been generally accepted that the permanganate method, when applied to mono-ethylenic acids, gives a result which is consistent with that obtainable by the ozonide method, and furthermore we have examined the constitution of hiragonic acid having three ethylenic linkings by the permanganate and the ozonide methods, and have found that the both methods gave consistent results. Hence the difference of the oxidation methods alone can not account for the marked discrepancies between the results obtained by these authors and those obtained by us.(5)

It is a very remarkable fact that, as it is seen from our studies hitherto reported, the highly unsaturated acids, not only clupanodonic acid but also eicosatetraenoic, moroctic and hiragonic acids, have no ethylenic linking in the same positions as the corresponding mono-ethylenic acids as shown in Table 2.

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(5) After the experiments given in this paper had been completed, there appeared another paper by Inoue and Kato (*Proc. Imp. Acad. Japan*, **10** (1934), 463) in which they maintained their formula having a triple linking. Also Kino (*Bull. Inst. Phys. Chem. Research, Japan*, **13** (1934), 1065) touched upon the constitution of clupanodonic acid in his studies on the polymerisation of highly unsaturated acids.

Table 2.

Number of carbon atoms	Highly unsaturated acids		Mono-ethylenic acids	
22	Clupanodonic	4:5, 8:9, 12:13, 15:16, 19:20	Cetoleic	11:12
20	Eicosatetraenoic	4:5, 8:9, 12:13, 16:17	Gadoleic	9:10
18	Moroctic	4:5, 8:9, 12:13, 15:16	Oleic	9:10
16	Hiragonic	6:7, 10:11, 14:15	Zoomaric	9:10

Judging from the relations between the constitutions of linolenic, linoleic and oleic acids found in vegetable oils, it would seem that one of the ethylenic linkings in the highly unsaturated acids in marine animal oils should be located in the same position as those in the corresponding mono-ethylenic acids. No such relation was, however, found to exist in any of the four above-mentioned highly unsaturated acids in marine animal oils. This is of much interest in connection with the mechanism of the formation of unsaturated fatty acids in living organism.

Experimental.

1. **Ozonolysis of Amyl Clupanodonate.** The same sample of clupanodonic acid as described in the previous paper was used in these experiments. It had d_4^{15} 0.9390, n_D^{15} 1.5035, neutralisation value 170.4 (calc. 169.8), iodine value(6) 383.2 (calc. 384.2), and gave 156% of ether insoluble bromide having Br-content 70.88% (calc. for $C_{22}H_{34}O_2Br_{10}$: 70.76%). It was heated with an equal amount of amyl alcohol containing 2.5% of hydrogen chloride on the water-bath for 1 hour, and after removing the excess of amyl alcohol and some unchanged clupanodonic acid, the amyl clupanodonate was separated. This was dissolved in chloroform, 50 c.c. being used for 5 g. of amyl clupanodonate, cooled with ice-salt, and treated with ozonised oxygen until the solution became saturated with ozone. The solvent was distilled off, and the ozonide was obtained as a light yellow syrup. Since the ozonide was highly unstable and there was a danger of explosive decomposition on undue heating, the chloroform was not thoroughly distilled off, and consequently the ozonide obtained above retained some chloroform. The yield of ozonide thus obtained was 17 g. (170%) from 10 g. of amyl clupanodonate. The theoretical yields for normal ozonide $C_{27}H_{44}O_{17}$ and ozonide peroxide $C_{27}H_{44}O_{18}$ are 159.9 and 163.9% respectively. Water (100 c.c.) was added to the ozonide, and the liquid was heated in a flask on the water-bath for 30 minutes. The flask containing the ozonide was attached by a delivery tube to other three flasks (**a**, **b** and **c**) which were connected in succession, the first (**a**)

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(6) Unless otherwise stated, the iodine values recorded in this paper were determined by the Wijs method.

being filled with ice-cold water, and the second (**b**) and the third (**c**) being filled with approximately 1/3 N solution of barium hydroxide. During the heating, a current of hydrogen was passed into the flask containing the ozonide, and the volatile substances (**A**) formed by the decomposition of ozonide were carried over with hydrogen into the three flasks (**a**, **b** and **c**) where they were absorbed by water and barium hydroxide solution in succession. The decomposition products remained in the initial flask partly separated as an insoluble oil (**C**) from the aqueous solution. The aqueous solution was saturated with sodium chloride, and extracted with 1 l. of ether which dissolved the decomposition products (**B**) contained in the aqueous solution. After distilling off ether from the ethereal solution, there remained the decomposition products (**B**) as a reddish orange liquid. The products of ozonolysis were thus separated into three portions (**A**, **B** and **C**).

(i) *Volatile products* (**A**). The aqueous solution in the flask (**a**) produced a pink colouration with Schiff's reagent, and a deep blue colouration with diethylamine and sodium nitroprusside, indicating the presence of acetaldehyde. The presence of propyl aldehyde was also indicated by the smell of scatol which was recognised by heating the phenylhydrazone, prepared from the aqueous solution in the flask (**a**), with zinc chloride to 180°. A partial separation of these two aldehydes was effected by heating the solution in the flask (**a**) at about 45° in a current of carbon dioxide. The flask was connected with another flask containing ice-cold water by a delivery tube, and the volatilised substances which were carried over with carbon dioxide were collected in the latter. By these treatments, the substances, dissolved in the aqueous solution in the flask (**a**) were separated into the portion (**a$_1$**) which did not volatilise at 45° and the portion (**a$_2$**) which volatilised at 45°. The *p*-nitrophenylhydrazone prepared from the portion (**a$_1$**) melted at 122.5–123° after recrystallisation from 50% alcohol; it was found to consist mainly of *p*-nitrophenylhydrazone of propyl aldehyde (Found: N, 21.82. Calc. for $C_9H_{11}O_2N_3$: N, 21.76%). The same derivative prepared from the portion (**a$_2$**) melted at 116–117° after recrystallisation from 50% alcohol; it was considered to be a mixture of *p*-nitrophenylhydrazones of acetaldehyde and propyl aldehyde (Found: N, 22.97. Calc. for $C_8H_9O_2N_3$: N, 23.46. Calc. for $C_9H_{11}O_2N_3$: N, 21.76%). The solution in the flask (**a**) showed also an acid reaction and produced a deep red colouration on adding ferric chloride after neutralisation, from which the presence of acetic acid was inferred.

The barium hydroxide solution in the flask (**b**) was found to contain a large amount of a white precipitate which was also found in the flask (**c**) in a small amount. This precipitate was found to be barium carbonate, indicating that carbon dioxide was formed by the ozonolysis of amyl clupanodonate. Calculating from the quantity of barium carbonate, the yield of carbon dioxide was found to be 0.81 g. or 8.1% of amyl clupanodonate used for ozonolysis. If amyl clupanodonate has the group $=CH \cdot CH_2 \cdot CH=$ and the products of ozonolysis derived from this group undergo a secondary decomposition with the formation of carbon dioxide to a quantitative extent, the yield of carbon dioxide should be 10.99% of amyl clupanodonate.

(ii) *Products obtained from the aqueous solution* (**B**). Yield 7.7 g. These were boiled out three times with petroleum ether, using 50 c.c. each time. The portion which remained insoluble was a reddish orange liquid with little crystalline deposit at room temperature. Oxidation of this portion with an alkaline solution of potassium permanganate and subsequent acidification yielded a product consisting largely of a crystalline solid. This was spread on a sheet of filter paper in order to remove the liquid portion by means of absorption, and the remaining solid gave, after being washed with a little cold ether and then recrystallised from ethyl acetate, succinic acid which had neutralisation value 947.6 (calc. for $C_4H_6O_4$: 950.6) and melted at 182–183°, both alone and after admixture with a pure specimen of succinic acid. On removing the solvent from the petroleum ether extract, there remained a reddish orange liquid which on distillation gave 0.5 g. of a colourless distillate at 120–180° of the bath-temperature. The distillate thus obtained showed an acid reaction, and the silver salts prepared from it were found to be a mixture of silver acetate and silver propionate by analyses (Found for 1st crop of silver salt: Ag, 61.31. Found for 2nd crop of silver salt: Ag, 63.25. Calc. for $C_2H_3O_2Ag$: Ag, 64.64. Calc. for $C_3H_5O_2Ag$: Ag, 59.63%).

(iii) *Oily substances* **(C)**. Yield 3.7 g. Oxidation of these substances with alkaline permanganate followed by acidification yielded an acid ester which had neutr. value 308.1 and saponif. value 595.5 and was deemed to consist mainly of amyl hydrogen succinate (calc. for $C_9H_{16}O_4$: neutr. value 298.2, saponif. value 596.5). The free acid liberated from this acid ester in the usual way yielded, after recrystallisation from ethyl acetate, a pure succinic acid having neutr. value 948.0, m.p. and mixed m.p. 182–183°.

2. **Ozonolysis of Dibromo-derivative of Clupanodonic Acid.** For the preparation of the dibromo-derivative of clupanodonic acid, 15 g. of the acid was dissolved in 150 c.c. of ether, cooled with ice-salt, and the bromine solution prepared by dissolving 7.5 g. (calc. for the formation of dibromo-derivative: 7.26 g.) of bromine in 15 c.c. of glacial acetic acid was gradually added with constant stirring. After all the bromine solution has been added, the ethereal solution was allowed to stand for 2 hours. No insoluble bromide separated. The ethereal solution was washed with water, then dehydrated, and on distilling off the ether, there remained 22 g. of oily bromides. These were dissolved in 10 volumes of alcohol, and fractionally precipitated by adding a little water in each time. The yield and the iodine value of each precipitate are given in Table 3.

Table 3.

Precipitate	Yield (g.)	Iodine value
1st ppt.	1.5	120.7
2nd ppt.	12	209.2
3rd ppt.	3	209.8
Bromide remaining in the final filtrate	4	230.5

The 2nd and 3rd precipitates were united and dissolved in alcohol, and after fractional precipitation as before, the dibromo-derivative was finally obtained as an oily substance which showed iodine value 208.7 (calc. for $C_{22}H_{34}O_2Br_2$: 207.2). The dibromide (10.5 g.) thus obtained was ozonised in a chloroform solution. The resulting ozonide was decomposed by heating with water in the usual way. The volatile decomposition products (**A**) formed were passed through the water in the flask (**a**) and then through the barium hydroxide solution in the two flasks (**b** and **c**) in succession. The decomposition products, which did not volatilise, partly dissolved in water and oily substances (C) separated under the aqueous layer. The latter was thoroughly extracted with ether, and the ethereal extract yielded, on distilling off the ether, the decomposition products (B) initially dissolved in water.

(i) *Volatile decomposition products* (A). The aqueous solution in the flask (a) gives a deep blue colouration with diethylamine and sodium nitroprusside, indicating the presence of acetaldehyde. The *p*-nitrophenylhydrazone prepared from this solution yielded, after recrystallisation from 50% alcohol, yellow needles melting at 123–124°, which appeared to consist mainly of the *p*-nitrophenylhydrazone of acetaldehyde, since the melting point was not lowered on admixture with a pure specimen (m.p. 128°) of the latter in various proportions (Found: N, 23.17. Calc. for $C_8H_9O_2N_3$: N, 23.46%). A somewhat lower melting point compared with the pure specimen is to be attributed to the contamination with a minor amount of other ingredient, possibly *p*-nitrophenylhydrazone of propyl aldehyde. Hence the dibromo-derivative used for ozonolysis was likely not to be an individual compound in a state of purity; it contained, besides the main constituent which yielded acetaldehyde on ozonolysis, a minor amount of the bromo-derivative which yielded other aldehyde, such as propyl aldehyde, on ozonolysis.

The barium hydroxide solution in the flasks (b) and (c) was found to contain a precipitate of barium carbonate indicating the formation of carbon dioxide by ozonolysis. Calculating from the quantity of barium carbonate, the yield of carbon dioxide was found to be 6.6% of the dibromo-derivative used for ozonolysis. Assuming that the dibromo-derivative has one of the group $=CH\cdot CH_2\cdot CH=$, and carbon dioxide is formed to a quantitative extent by the secondary decomposition of the products of ozonolysis derived from that group, the yield of carbon dioxide is calculated to be 8.98% of the dibromo-derivative.

(ii) *Decomposition products obtained from aqueous solution* (B). These were a reddish orange liquid. Oxidation with alkaline permanganate followed by acidification yielded succinic acid which showed, on recrystallisation from ethyl acetate, neutr. value 949.1 and m.p. 182–183°.

(iii) *Oily substances* (C). Yield about 6 g. In order to eliminate the bromine from oily substances, these were dissolved in 10 c.c. of methanol and 6 g. of zinc powder was added in several portions. The mixture was refluxed on the water-bath, 10 c.c. of 5 N solution of hydrogen chloride in methanol was gradually added, and the debrominated product was taken up with ether. On removal of the solvent from the ethereal solution, the residue was heated in an oil-bath and there was obtained 1.4 g. of colourless liquid boiling below 180°. This was saponified, the unsaponifiable matter removed by extraction with ether and the free acid liberated. It had neutr. value 435.7 and iodine value 182.7 (calc. for $C_7H_{12}O_2$: neutr. value 438.0, iodine value 198.2), and was considered to consist mainly of heptenoic acid. The hydrogenation product was shown to be heptoic acid by preparing its barium salt (Found: Ba, 34.69. Calc. for $(C_7H_{13}O_2)_2Ba$: Ba, 34.73%).

3. Ozonolysis of Tetrabromo-derivative of Clupanodonic Acid. Fifteen g. of clupanodonic acid was dissolved in 150 c.c. of ether, and on cooling, the bromine solution prepared by dissolving 15 g. (calc. for the formation of tetrabromide:

14.52 g.) of bromine in 30 c.c. of glacial acetic acid was added with constant stirring. No precipitate was formed after standing for 2 hours. The ethereal solution was washed with water, dehydrated, and on removal of ether there remained 29.5 g. of bromides. These were dissolved in 10 volumes of alcohol, a minute amount of insoluble bromides was removed by filtration, and the filtrate was admixed with water so as to effect a fractional precipitation of the bromides. The yield and the iodine value of each precipitate are shown in Table 4.

Table 4.

Precipitate	Yield (g.)	Iodine value
1st ppt.	4	68.1
2nd ppt.	11	117.2
3rd ppt.	9.5	119.5
4th ppt.	0.5	119.1
Bromide remaining in the final filtrate	2.5	142.5

The 2nd and 3rd precipitates were combined, and subjected again to the fractional precipitation from alcoholic solution by adding water, by which a tetrabromo-derivative having iodine value 118.0 (calc. for $C_{22}H_{34}O_2Br_4$: 117.2) was separated. Sixteen g. of this bromide was subjected to ozonolysis in the same manner as described above in the case of amyl clupanodonate and dibromo-derivative. The volatile decomposition products (**A**) were made to be absorbed by water in the flask (**a**) and then by barium hydroxide solution in two flasks (**b** and **c**). The decomposition products, which did not volatilise, were separated into two portions: the decomposition products obtained from aqueous solution (**B**) and the oily substances (**C**).

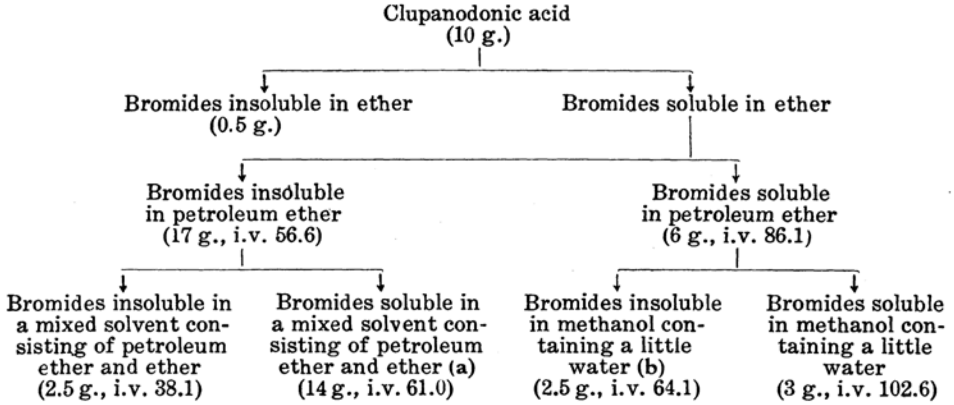
(i) *Volatile decomposition products* (**A**). The aqueous solution in the flask (**a**) showed an aldehyde reaction, and gave a small amount of precipitate on adding *p*-nitrophenylhydrazine. The barium hydroxide solution in the flask (**b**) was found to contain a small amount of the precipitates of barium carbonate, from which the yield of carbon dioxide formed by the ozonolysis was calculated to be 0.69% of the bromo-derivative used for ozonolysis. It is thus seen that volatile aldehyde and carbon dioxide were formed by the ozonolysis, but the yields of these compounds were so small that they were considered to be derived from a minor ingredient contaminating the tetrabromo-derivative, and not from the main constituent.

(ii) *Decomposition products obtained from aqueous solution* (**B**). These were a reddish orange liquid. Oxidation with alkaline permanganate followed by acidification yielded a crystalline product which, after being washed with a little ether, showed m.p. 177–178°, and recrystallisation of this product from ethyl acetate gave succinic acid having neutr. value 945.1 and m.p. 182–183°.

(iii) *Oily substances* (**C**). Yield 11.5 g. These were debrominated with zinc powder and hydrogen chloride in methanol. The debrominated product was distilled, and a fraction boiling in the neighbourhood of 130°/20 mm. was collected separately. This fraction was hydrogenated, saponified, freed from the neutral matter, and the acidic matter was separated which, recrystallised from dilute alcohol, showed neutr. value 324.1 (calc. for $C_{10}H_{20}O_2$: 325.9) and m.p. 29.5–30°. The melting point was not lowered when the substance was admixed with a pure specimen of capric acid, m.p. 30.5–31°, in various proportions.

4. **Ozonolysis of Hexabromo-derivative of Clupanodonic Acid.** Ten g. of clupanodonic acid was dissolved in 100 c.c. of ether, and on cooling, the bromine solution prepared by dissolving 15 g. (calc. for the formation of hexabromide: 14.52 g.) of bromine in 30 c.c. of glacial acetic acid was added gradually with constant stirring. After standing for 2 hours the precipitate of insoluble bromides formed in a minute amount was removed by filtration, and the soluble bromides in an ethereal solution was obtained on distilling off ether. These were subjected to a further separation by means of the solvents as shown in Table 5.

Table 5.



The bromides (**a**) and (**b**) were united and treated again with a mixed solvent consisting of petroleum ether and ether, the insoluble portion was removed, the soluble portion was further treated with methanol containing a little water, the soluble portion was discarded; the insoluble portion thus obtained had iodine value 62.0 (calc. for $C_{22}H_{34}O_2Br_6$: 62.7) and it was considered to consist of a hexabromo-derivative.

The hexabromo-derivative (11.5 g.) thus obtained was ozonised in chloroform solution and the ozonide was decomposed with water. Volatile decomposition products were not formed, and the decomposition products were separated into two portions as before, i.e., the products obtained from the aqueous solution and the oily substances. The former consisted of a reddish orange liquid which deposited a crystalline solid at room temperature. Oxidation with alkaline permanganate followed by acidification gave a product which, after being washed with a little cold ether, melted at 178–179° and yielded succinic acid on recrystallisation from ethyl acetate; neutr. value 948.2, m.p. 182–183°. The oily substances (9.5 g.) were debrominated with zinc powder and hydrogen chloride in methanol. Distillation of the debrominated product (2.7 g.) gave 1.3 g. of a distillate boiling below 170°/15 mm. This was hydrogenated and saponified, and the product was separated into acidic and neutral portions in the usual way. The acidic portion, after being recrystallised from 85% alcohol, was shown to be myristic acid $C_{14}H_{28}O_2$; neutr. value 243.7 (calc. 245.8), m.p. and mixed m.p. 53.5–54°. The neutral portion, after being treated with sodium bisulphite solution, showed acetyl value 216.9 (calc. for tetradecanol $C_{14}H_{30}O$: 219.0) which indicated that it consisted chiefly of higher alcohol. The free alcohol liberated from the acetylated product melted at 30.5–31° after recrystallisation from 85% alcohol. This is considered to consist chiefly of tetradecanol formed by the hydrogenation of the corresponding aldehyde which is likely to be present in the debrominated product, but its melting point is considerably lower than that of pure tetradecanol. Krafft(7) gives m.p. 38° for the same compound.

Summary.

1. Amyl clupanodonate was subjected to ozonolysis. Among the products of ozonolysis were found propyl aldehyde, acetaldehyde, carbon dioxide, succinic acid, amyl hydrogen succinate and also lower acids which were deemed to consist of propionic and acetic acids. Of these compounds, acetaldehyde, acetic acid and carbon dioxide are attributable to the secondary decomposition of the products of ozonolysis primarily derived from the group $=CH{\cdot}CH_2{\cdot}CH=$. Accordingly clupanodonic acid was shown to contain the following groups: $CH_3{\cdot}CH_2{\cdot}CH=$, $=CH{\cdot}(CH_2)_2{\cdot}COOH$, $=CH{\cdot}CH_2{\cdot}CH=$ and $=CH{\cdot}(CH_2)_2{\cdot}CH=$, of which clupanodonic acid contains three of the last named group.

2. The dibromo-derivative of clupanodonic acid was separated as the chief constituent of the product which was obtained by adding 1 mol bromine to 1 mol clupanodonic acid. Similarly tetrabromo- and hexabromo-derivatives of clupanodonic acid were separated, respectively, as the chief constituents of the product obtained by adding 2 mols and 3 mols bromine, respectively, to 1 mol clupanodonic acid. It is seen from the following results obtained by the ozonolysis of these bromo-derivatives that, in these partial brominations, bromine adds selectively first to the ethylenic linking which is more distant from the carboxyl group. Though this selectivity is not a complete one, any of the above-mentioned bromo-derivatives contains as its chief constituent the product formed in accordance to this selectivity.

3. Ozonolysis of dibromo-derivative of clupanodonic acid thus obtained gave a bromo-compound which on debromination and subsequent hydrogenation yielded, as an acidic product, heptoic acid. This fact, coupled with the results obtained by the ozonolysis of amyl clupanodonate, indicated that clupanodonic acid had the group
$CH_3{\cdot}CH_2{\cdot}CH=CH{\cdot}(CH_2)_2{\cdot}CH=$.

4. Ozonolysis of tetrabromo-derivative of clupanodonic acid gave a bromo-compound which on debromination and subsequent hydrogena-

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(7) *Ber.*, (1883), 1714.

tion yielded capric acid, and consequently clupanodonic acid was shown to contain the group $CH_3\cdot CH_2\cdot CH{=}CH\cdot(CH_2)_2\cdot CH{=}CH\cdot CH_2\cdot CH{=}$.

5. Ozonolysis of hexabromo-derivative of clupanodonic acid gave a bromo-compound which on debromination and subsequent hydrogenation yielded myristic acid, and consequently clupanodonic acid was shown to contain the group $CH_3\cdot CH_2\cdot CH{=}CH\cdot(CH_2)_2\cdot CH{=}CH\cdot CH_2\cdot CH{=}CH\cdot(CH_2)_2\cdot CH{=}$.

6. From the foregoing results, the constitution of clupanodonic acid has been established as $\Delta^{4:5,\,8:9,\,12:13,\,15:16,\,19:20}$-docosapentenoic acid which is expressed by the following formula:

$$CH_3\cdot CH_2\cdot CH{=}CH\cdot(CH_2)_2\cdot CH{=}CH\cdot CH_2\cdot CH{=}CH\cdot(CH_2)_2\cdot CH{=}CH\cdot(CH_2)_2\cdot CH{=}CH\cdot(CH_2)_2\cdot COOH.$$

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