ON THE CHEMICAL CONSTITUTION OF CLUPANODONIC ACID.

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The unsaturated acids occurring in natural oils and fats, which have hitherto been investigated by chemists for their chemical constitution, are chiefly those of the oleic acid series. Of the acids of higher unsaturation we may count only linolic, elaeostearic, and linolenic acids. As to the so-called highly unsaturated acids ($C_nH_{2n-8}O_2$ and $C_nH_{2n-10}O_2$ series), which occur commonly and abundantly in marine animal oils, no reliable investigation has yet been performed, and at present we lack nearly wholly the definite knowledge of their constitution. The present paper deals with the constitution of clupanodonic acid, $C_{22}H_{34}O_2$, which is one of the most important and typical members of such series.

More than thirty years ago W. Fahrion[1] reported that on oxidising "jecoric acid", $C_{18}H_{30}O_2$, by Hazura's oxidation method he was unable to obtain hydroxylated acids, except volatile acids and carbon dioxide. Presumably his "jecoric acid" was impure highly unsaturated acids. R. Majima and T. Okada[2] attempted to investigate the constitution of clupanodonic acid, but the acid obtained by debrominating the polybromide of the fatty acids of Japanese sardine oil was found not to be a simple chemical compound; so they were obliged to gave up their original project. Recently from a study on the fatty acids of a "South Georgia whale oil", E. F. Armstrong and T. P. Hilditch[3] made an interesting statement that in C_{20} and C_{22} acid groups was there slightest evidence of the occurrence of an ethylenic linkage between the carboxyl group and the ninth carbon atom in the chain. This conclusion appears, however, to be too hasty, inasmuch as they did not isolated the C_{20} and C_{22} highly unsaturated acids, but oxidised the mixed acid fractions respectively. But there must have been present a large amount of less unsaturated acids, such as $C_{20}H_{38}O_2$ (gadoleic acid ?) and cetoleic acid, $C_{22}H_{42}O_2$, in the fractions, whereas the amount of the highly unsaturated acids was small.[4] The statement propounded by R. R. M^{c} Gregor with G. D. Beal[5] on the limit of unsaturation in menhaden oil is

(1) *Chem. Ztg.*, **17** (1893), 521.
(2) *J. Tokyo Chem. Soc.*, **35** (1914), 13.
(3) *J. Soc. Chem. Ind.*, **44** (1925), 180 T.
(4) Y. Toyama, *Chem. Umschau*, **31** (1924), 221, 238; **33** (1926), 293; **34** (1927), 19.
(5) *J. Am. Chem. Soc.*, **48** (1926), 3150.

essentially an amplification of the conclusion of Armstrong and Hilditch. Nor were they able to arrive at definite results by the decomposition of the ozonides of the highly unsaturated acids.

The present writer has succeeded to isolate nearly pure clupanodonic acid by his lithium-salt-acetone method.[1] The method of bromination and debromination hitherto used for the isolation of polyethylenic acids, such as linolic and linolenic acids, has a drawback that there is no certain proof that the position of ethylenic linkage is not displaced by such violent chemical treatments. The comparatively mild treatments (saponification, esterification, distillation under diminished pressure) of the writer's method are probably free from the above defect.

Clupanodonic acid readily absorbs ozone in chloroformic solution forming ozonide. But this compound appears to be unstable and it has been found difficult to obtain it in pure state. On decomposing the ozonide with water, the nearly whole part of the decomposition products go to the solution leaving only a small amount of an oily insoluble substance. By the writer's experiments, there were found as volatile decomposition products acetaldehyde, propionaldehyde, and carbon dioxide, and as non-volatile product a comparatively large amount of succinic acid. Azelaic acid and other higher dibasic acids, or their semialdehydes were found not to be present, at least appreciably. According to Armstrong and Hilditch's statement, a marked amount of such compounds should be present in the decomposition products.

As the decomposition products of clupanodonic acid ozonide were mainly soluble in water, so for the confirmation of the product containing the carboxyl group, the amyl ester of the acid was ozonised and decomposed with water. By this means an insoluble decomposition product containing the esterified carboxyl group was obtained, which on saponification yielded succinic acid. So the product contained four carbon atoms; therefore the first ethylenic linkage in clupanodonic acid is situated between fourth and fifth carbon atoms counting from the carboxyl group. Acetaldehyde and carbon dioxide were likely formed as the secondary decomposition products of malonic dialdehyde, or semialdehyde of malonic acid. Now calculating from the amount of the carbon dioxide formed, the atomic group, $=CH\cdot CH_2\cdot CH=$, contained in a molecule of clupanodonic acid is probably one. Conjugate double bonds are probably not present, since neither glyoxal nor glyoxylic acid was detected, and also no trace of oxalic acid was found; also by heating clupanodonic acid with metallic sodium and alcohol, no lowering of the iodine value was observed. The presence of triple bonds is negated by the fact that the iodine value of clupanodonic acid and the

(1) *Chem. Umschau*, **27** (1920), 229; **33** (1926), 285.

bromine content of its bromine addition compound closely agree with the numbers calculated from five double bonds.

By the above mentioned results, it has been confirmed that the molecule of clupanodonic acid consists of the following atomic groups: (1) $CH_3{\cdot}CH_2{\cdot}CH=$, (2) $=CH{\cdot}CH_2{\cdot}CH=$, (3) $=CH{\cdot}(CH_2)_2CH=$, and (4) $=CH{\cdot}(CH_2)_2{\cdot}COOH$. As to some of the arrangement of these groups, no definite data have yet been obtained. But according to a recent investigation of Y. Toyama,[1] cetoleic acid, $C_{22}H_{42}O_2$, which occurs widely distributed in marine animal oils and has 22 carbon atoms alike to clupanodonic acid, has been proved to be $\Delta^{11,12}$ dococenic acid. From the fact that linolic and linolenic acids have each an ethylenic linkage between ninth and tenth carbon atoms as oleic acid, so if we assume an analogy exists in the case of clupanodonic and cetoleic acids, it is not unreasonable to think that clupanodonic acid has an ethylenic linkage between eleventh and twelfth carbon atoms as cetoleic acid. Then the possible arrangement of carbon atoms in the molecule of clupanodonic acid is $C_3=C_4=C_4=C_4=C_3=C_4$ (right end—carboxyl) and $C_3=C_4=C_4=C_3=C_4=C_4$ (ibid.), so that the constitution of clupanodonic acid will be one of the following two formulae :[2]

(1) $CH_3{\cdot}CH_2{\cdot}CH:CH{\cdot}(CH_2)_2CH:CH\,(CH_2)_2{\cdot}CH:CH{\cdot}(CH_2)_2\,CH:CH{\cdot}CH_2{\cdot}CH:CH\,(CH_2)_2{\cdot}COOH$.

(2) $CH_3{\cdot}CH_2{\cdot}CH:CH\,(CH_2)_2\,CH:CH{\cdot}(CH_2)_2\,CH:CH{\cdot}CH_2{\cdot}CH:CH\,(CH_2)_2{\cdot}CH:CH{\cdot}(CH_2)_2\,COOH$.

As the decomposition products of clupanodonic acid ozonide are very complicated, no pretension can be insisted for not overlooking some important compounds. And as some of the arrangement of the atomic groups is hypothetical, the above formulae have only been proposed provisionally. Further investigations may verify or correct them. As to the arrangement of the atomic groups, the examination of the decomposition products of partially hydrogenated clupanodonic acid may probably furnish some clue for its definite confirmation.

Experimental Part.

(I) **Material.** The fatty acids of a Japanese sardine oil from Hokkaidō were treated by the lithium-salt-acetone method, and the highly unsaturated acids so obtained were changed to the methyl esters and fractionated under 5 mm. pressure. The fraction boiling at 217–226° was taken as the methyl ester of clupanodonic acid. It was saponified and the accompanying small

(1) *Chem. Umschau*, 35 (1928), 20.

(2) That clupanodonic acid has straight chain structure is already known by the formation of behenic acid on hydrogenation.

amount of unsaponifiable matter was removed, and finally oxidised acids were separated by means of petroleum ether. The clupanodonic acid thus refined had the following properties: $d_4^{15}=0\ 9410$, neutralisation value 170.7, iodine value 388.8, $n_D^{15}=1.5057$. Calculated for $C_{22}H_{34}O_2$: neutr. value 169.8, iodine value 384.3.

The specific gravity and refractive index were respectively higher than those of previously obtained. Whether these are due to the presence of more highly unsaturated acids (e. g. $C_{22}H_{32}O_2$), or the previously isolated clupanodonic acid was still contaminated with less unsaturated acids, is not certain. In the present experiments the above sample was, however, accepted as pure clupanodonic acid.

(II) **Preparation of Clupanodonic Acid Ozonide.** Five gr. of clupanodonic acid were dissolved in 50 c.c. of chloroform, and on cooling with ice and salt a current of ozonised oxygen (washed with 5% NaOH solution and sulphuric acid; ozone content ca. 5%) was passed into the solution at the rate of 6 litres per hour during for 5 hours. Then the chloroform was distilled off under a diminished pressure at about 30°. By keeping the ozonide over night under diminished pressure it was decomposed with water. The yield of the ozonide was 11–12 gr. per 5 gr. of the acid, so that it was much higher than that calculated for normal ozonide (8.6 gr.). This was probably due to the difficulty of removing last part of the solvent. The ozonide appeared also to be unstable, so it was decomposed next day without further keeping.

The ozonide thus obtained formed a pale yellow, elastic, linoxyn-like mass, and burned explosively when heated. Great difficulty was experienced in its elementary analysis, and the result was very unsatisfactorily. The carbon content was found apparently much higher; this was probably caused by adhered chloroform.

(III) **Decomposition of Clupanodonic Acid Ozonide with Water.** To the ozonide obtained from 5 gr. of clupanodonic acid, 100 c.c. of water were added and heated on a water bath in the current of hydrogen. The flask was fitted with a condenser, and the latter in turn combined to the following three flasks, through which the evolved gas together with hydrogen may pass without leakage: (*a*) 50 c.c. of water (cooled with ice), (*b*) 150 c.c. of ca. N/3 $Ba(OH)_2$ solution, (*c*) 50 c.c. of the same. As the ozonide by rapid heating with water made an explosion-like decomposition, so the mixture was at first very carefully warmed to 65–70°, and kept somewhat long time at this temperature, and then the temperature was gradually raised till the water in the bath boiled. The ozonide decomposed with evolution of gas, firstly floated on the surface of water as a viscous oily liquid, this then gradually went to the solution, and finally there remained at the bottom of

the flask a small amount of a brownish insoluble liquid substance. The solution became discoloured by degrees and assumed a brownish orange-yellow colour. The above operation was five times repeated and so totally the ozonide obtained from 25 gr. of clupanodonic acid was decomposed.

(1) *Volatile Decomposition Products.* The volatile products caught in the flasks *a*, *b* and *c* were examined.

(*a*) The solution in the flask *a* showed marked aldehyde reactions. A colourless liquid collected at the bottom of the flask was found to be admixed chloroform. With *p*-nitrophenylhydrazine and hydrochloric acid the solution gave a bright orange-yellow precipitate, which on twice recrystallisations from 50% alcohol, formed orange-yellow needles of melting point 116.5–117°. 0.1138 gr. subst. gave 0.2308 gr. CO_2 and 0.0581 gr. H_2O; 0.1425 gr. subst. gave 28.9 c.c. nitrogen (23°, 756 mm.). (Found: C=55.31; H=5.71; N=22.61. $C_8H_9O_2N_3$ requires C=53.60; H=5.07; N=23.47. $C_9H_{11}O_2N_3$ requires C=55.93; H=5.74; N=21.76%). The percentage of carbon and hydrogen corresponded nearly to those of the nitrophenylhydrazone of propionaldehyde, but that of nitrogen was the mean of those of the compounds of acetaldehyde and propionaldehyde. The presence of the two aldehydes was proved by (1) diethylamine and sodium nitroprusside (acetaldehyde), and (2) skatole reaction (propionaldehyde).

(*b*) The precipitate formed in the flasks, *b* and *c*, appeared to consist of barium carbonate. The carbon dioxide was possibly formed as the secondary decomposition product of the ozonide. For its quantitative determination, the following procedure was adopted. The excess of $Ba(OH)_2$ was firstly neutralised with N/2 hydrochloric acid using phenolphthalein as indicator. Then adding methylorange as indicator the further addition of hydrochloric acid was made until the white precipitate was dissolved and the solution assumed a clear pink colour. From the amount of HCl used for the second titration, the weight of carbon dioxide was calculated.

The determination was repeated with the ozonide obtained from each 5 gr. of clupanodonic acid. As the most reliable number, the writer obtained 0.838 gr., i.e. ca. 16.8% for clupanodonic acid. The presence of acetaldehyde and carbon dioxide in the decomposition products of the ozonide is, as already stated, probably due to the secondary decomposition of malonic dialdehyde, or semialdehyde of malonic acid. So it may be inferred that in the molecule of clupanodonic acid there are contained atomic groups, such as $=CH\cdot CH_2\cdot CH=$ or $=CH\cdot CH_2\cdot COOH$. If there is only one such group, $=CH\cdot CH_2\cdot CH=$ (40) in one molecule of clupanodonic acid (330.3), then the carbon dioxide formed by the decomposition ($CH\cdot CH_2\cdot CH \rightarrow CO_2 + CH_3\cdot CHO$) must be 13.3% for clupanodonic acid. The number obtained above (16.8%) was somewhat greater than it, but if we assume the presence of two such

groups, then the amount of carbon dioxide is too small, so that the number of the group contained is probably only one. That the carboxyl is not contained in the group was confirmed by the experiments made on the ozonide of the amyl ester of clupanodonic acid (see below).

(2) *Non-Volatile Decomposition Products.* The aqueous solution of the decomposition products obtained from each 5 gr. of clupanodonic acid ozonide was treated as follows:

Firstly the solution was filtered through a filter paper, thereby the insoluble substance was left. The filtrate was twice treated with each 150 c.c. of ether, the ether layer separated from the lower aqueous solution (*C*), and treated with sodium bicarbonate solution in a separating funnel. The upper ether solution was separated, dehydrated with anhydrous magnesium sulphate and the ether distilled off. The thereby remaining portion (*A*) consisted chiefly of aldehydic constituents. The sodium bicarbonate extract was acidified with dilute hydrochloric acid and exhausted with ether (three times with each 100 c.c.) and then ether distilled off; by this operation the acidic constituents (*B*) were obtained. The yield of the products was found difficult to determine exactly, chiefly owing to their volatility. But roughly speaking the following numbers were obtained:

From the ozonide of 10 gr. of clupanodonic acid: *A* only 0.3 gr.; *B* 3.2 gr.; *C* or the aqueous solution separated from ether layer was evaporated under a diminished pressure, and about 5 gr. of the residue was obtained. On the evaporation the solution became discoloured, and a substance difficultly soluble in ether or water was produced. This was probably the resinified products of aldehydes. The insoluble oily substance firstly formed was about 0.6 gr.

(a) Part *A*. On distilling off the ether, an orange-yellow liquid was left. This was difficultly soluble in water; it formed with nitrophenylhydrazine a dark reddish viscous precipitate, and with semicarbazide hydrochloride a semicarbazone (?) of m.p. above 200°. As no definite derivative was obtained, the substance was analysed as follows: 0.1373 gr. subst. gave 0.3033 gr. CO_2 and 0.0946 gr. H_2O. (Found C=60.25, H=7.71%). If we consider the substance to be an aldehyde or its polymerised product, so succinic dialdehyde $C_4H_6O_2$ (C=55.78, H=7.03%) or glutaric dialdehyde $C_5H_8O_2$ (C=59.96, H=8.06%) correspond somewhat to it.

(b) Part *B*. From the ozonide of 25 gr. of clupanodonic acid, about 8 gr. of this part was obtained. It formed an orange-yellow liquid not solidified by cooling in ice. It was firstly distilled at the ordinary pressure, thereby at the bath temperature of 140° the distillation began. At the bath temperature of 160° (dist. temp. ca 110°) the distillation was interrupted. The distillate (*B*1) amounted to 1.9 gr.; a pale yellow pungent liquid of

$n_D^{20}=1.3700$ and of aldehydic reaction. Then the distillation was continued under 6 mm. pressure. Up to the bath temp. of 180° and the distillation temp. of 135°, 0.8 gr. of distillate (*B*2) was obtained; this was a pale orange-yellow liquid. By this temperature, the indication of decomposition was observed; so the distillation was stopped. The residue (*B*3), 4.2 gr.; a dark brown viscous liquid mixed with crystals.

*B*1. This was dissolved in alcohol and titrated with $Ba(OH)_2$ solution (acid value found was 520); it was then exhausted with ether to remove aldehydic compounds. The barium salt obtained by evaporating the alcohol and water contained 55.4%Ba, ($(C_2H_3O_2)_2Ba$ requires Ba=53.79%); so the acid seemed to consist of acetic acid. But judging from somewhat high distillation temperature, there appeared the presence of propionic acid (b.p. 141°) to be likely.

*B*2. Showed a marked aldehyde reaction and had the acid value 425. On oxidising it with alkaline potassium permanganate solution, white crystals were obtained. On carefully washing with ether, they melted at 177.5° and the mixed melting point test with succinic acid[1] showed 180–181°. So this substance was confirmed to be succinic acid. The crude product probably contained succinic semialdehyde.

*B*3. Showed also a marked aldehyde reaction. By treating it with ether, a brown tarry insoluble substance was left; it was, however, completely soluble in acetic ether. On oxidising it with alkaline potassium permanganate solution, a yellowish white crystalline semisolid was obtained with a yield of 70%; the white crystals obtained by treating it with ether to remove the liquid part melted at about 177° and the mixed test with succinic acid was 179–180°. So the substance was confirmed to be also succinic acid.

(c) Part *C*. On distilling off the ether under a diminished pressure, a dark brownish viscous substance was obtained. It was soluble in acetone and acetic ether, but on treatment with ether, a black-brown viscous substance was left. This was probably polymerised aldehydes. As the substance did not distil under diminished pressure when heated to a bath temperature of 150°, so the content of the flask was dissolved out with acetone (thereby much amount of tarry substance was separated). The substance left on evaporating off the acetone was dissolved in a little water and exhausted with ether. On evaporating off the ether, an extract was obtained with a yield of about 54%. This was a mixture of crystals and an orange-yellow liquid. It was then treated with cold ether to remove the liquid part, and the crystals were dissolved in acetic ether, and decolourised with animal charcoal. On concentration and cooling, the solution deposited white crystals,

(1) Merck, m. p. 183-183.5°. In this and in the following experiments, always equal parts were mixed.

which melted at 182–182.5° and had the neutralisation value 936. The mixed test with succinic acid was 182°. So the substance was succinic acid.

On directly oxidising the original substance with alkaline potassium permanganate solution, white crystals of m.p. 182–182.5° (mixed test with succinic acid 181.5–182°) and the neutr. value 934 were obtained. Its elementary analysis gave the following result: 0.1187 gr. subst. gave 0.1786 gr. CO_2 and 0.0574 gr. H_2O. (Found: C=41.04; H=5.41. $C_4H_6O_4$ requires C=40.66; H=5.12%). The substance was, therefore, confirmed to be succinic acid.

Beside the above mentioned experiments the insoluble oily substance, which was formed in a small amount on decomposing the ozonide with water, was oxidised, and a substance (white crystals) resembling azelaic acid was obtained, but owing to its small quantity it was found difficult to confirm it with certainty. If this was really azelaic acid, then the oily substance was probably formed from ozonides of some less unsaturated acids, which were contaminated in the sample of clupanodonic acid.

(IV) **Decomposition of the Amyl Ester Ozonide of Clupanodonic Acid.** This experiment was undertaken to confirm the position of the ethylenic linkage nearest to the carboxyl group. As the decomposition products of clupanodonic acid ozonide with water are nearly wholly soluble in water, in order to settle the question the writer has esterified the acid with a sparingly soluble alkyl group and ozonised it. By decomposition with water, the atomic group containing the esterified carboxyl group will be obtained as an insoluble product and its nature may be identified. Apparently a higher alkyl group would be better for the purpose, but as it is difficult to expel it, so amyl group was chosen for the experiment.

Six gr. of clupanodonic acid, 24 gr. of amyl alcohol containing 2.5% of HCl and 30 c.c. of ether were heated in a flask with a reflux condenser on a water bath in the current of carbon dioxide for one hour and then the content of the flask was washed with water, taken up with ether, and dried with anhydrous sodium sulphate. The ether was then distilled off and the excess of amyl alcohol was removed under a diminished pressure. The amyl ester so prepared had the acid value of 9.9 showing some presence of free acid, but as it had the saponification value 139.5, nearly corresponding to that (140.1) of amyl clupanodonate, $C_{21}H_{33}COOC_5H_{11}$, it was used as the material for ozonisation.

Five gr. of the amyl ester were dissolved in 50 c.c. of chloroform and ozonised. The yield of the ozonide, which contained still some amount of the solvent, was 9.8 gr. It was an orange-yellow viscous liquid. On heating it with 80 c.c. of water on a water bath for one hour, there was observed an appreciable amount of an orange-yellow insoluble oily liquid formed at the

bottom of the flask. The supernatant liquid was filtered off and the oily substance was again heated with 8 times of water in order to complete the decomposition. The oily substance finally left was 2.46 gr. corresponding to about 49% of the original ester.

This substance was dissolved in alcohol and titrated with alcoholic potash to neutralisation, and then on adding water the aldehydic or neutral substances were extracted with ether, and the aqueous solution was acidified with hydrochloric acid and exhausted with ether. On distilling off the ether, about 32% of a dark brownish liquid of the neutralisation value 255 was obtained (neutr. value of mono-amyl ester of succinic acid $HOOC(CH_2)_2 \cdot COOC_5H_{11} = 298.2$). Then the substance was saponified with an excess of alcoholic potash and acidifying with hydrochloric acid the free acid was extracted with ether. The extract consisted of a mixture of liquid and crystals. This was treated with ice-cooled ether to remove the liquid part and then the crystals were dissolved in acetic ether and decolourised with animal charcoal. On concentrating the solution and cooling with ice, crystals were obtained, which melted at 177° and had the neutralisation value 605. As this seemed still to contain some amount of neutral substances, so the neutralised solution was evaporated to dryness, dissolved in water and treated with ether. To the aqueous solution, dilute hydrochloric acid was added, and the free acid was exhausted with ether. The solid remaining on evaporating off the ether melted at 179–180° and had the neutr. value 944. The mixed test with succinic acid was 181–181.5°. So this substance was identified as succinic acid.

Then the firstly extracted aldehydic part (ca. 42%) was examined. It was oxidised with an alkaline potassium permanganate solution and the product was neutralised with alcoholic potash, and the neutral substances extracted with ether. From the lower aqueous solution on decomposing it with hydrochloric acid there was obtained by exhausting with ether a substance probably consisting of mono-amyl ester of succinic acid. It was a pale yellow liquid of the neutr. value 294. On saponification the free acid was obtained; it was refined by means of acetic ether. The substance formed white crystals of m.p. 180. The mixed test with succinic acid was 181–181.5; so this was also succinic acid.

From the above results it has been concluded that among the decomposition products of the ozonide of amyl clupanodonate, that part which is combined with the amyl group is succinic acid. Therefore the ethylenic linkage nearest to the carboxyl group in the molecule of clupanodonic acid is situated between fourth and fifth carbon atoms counting from the carboxyl group. The calculated percentage of succinic mono-amyl ester to be obtained from amyl clupanodonate is 47%. The yield of 49% nearly corresponded to it.

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