THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL.
IX. THE CONSTITUTION OF DOCOSAHEXENOIC ACID $C_{22}H_{32}O_2$.

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In previously reported studies⁽¹⁾ on the highly unsaturated C_{22}-acids in sardine oil, clupanodonic acid $C_{22}H_{34}O_2$ was isolated in much purer state than obtained before, and its constitution was established as $\Delta^{4:5,\,8:9,\,12:13,\,15:16,\,19:20}$-docosapentenoic acid. A concentrated fraction of docosahexenoic acid $C_{22}H_{32}O_2$ was also separated. The present paper records the experimental results obtained by the ozonolysis of the amyl ester prepared from the concentrated fraction of docosahexenoic acid.

Among the products of ozonolysis acetaldehyde $CH_3\cdot COH$, acetic acid $CH_3\cdot COOH$, carbon dioxide CO_2, succinic acid $HOOC\cdot(CH_2)_2\cdot COOH$ and amyl hydrogen succinate $HOOC\cdot(CH_2)_2\cdot COOC_5H_{11}$ were found; the presence of the aldehydes corresponding to succinic acid and amyl hydrogen succinate was also indicated. Although the amyl ester used for ozonolysis was contaminated with a small proportion of

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(1) This Bulletin, **10** (1935), 433, 441.

amyl clupanodonate which yielded on ozonolysis the above compounds as shown in a preceding paper,(2) the yields of the above compounds obtained in these experiments indicated that they owed their origin largely to amyl docosahexenoate which constituted the bulk of the amyl ester used for ozonolysis. The volatile aldehydes formed by the ozonolysis appeared not to consist exclusively of acetaldehyde, but no other volatile aldehydes than acetaldehyde were identified.(3) Of the products of ozonolysis obtained in these experiments, 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, whilst carbon dioxide is formed, together with acetaldehyde and acetic acid, by the secondary decomposition of the products of ozonolysis derived from the group $=CH\cdot CH_2\cdot CH=$, and the yield of carbon dioxide indicated the presence of at least three of the group $=CH\cdot CH_2\cdot CH=$ in amyl docosahexenoate. Accordingly the free docosahexenoic acid was found to contain the following groups: $=CH\cdot(CH_2)_2\cdot CH=$, $=CH\cdot CH_2\cdot CH=$ (at least three), and $=CH\cdot(CH_2)_2\cdot COOH$. Although the group attached to the opposite side of the carboxyl group was left undetermined in these experiments, it must be $CH_2=$ or $CH_3\cdot CH=$ depending on whether docosahexenoic acid has three or four of the group $=CH\cdot CH_2\cdot CH=$, since it has 22 carbon atoms in total. Hence, docosahexenoic acid is composed of either one of the following two sets of the groups:

(1) $CH_2=$, $=CH\cdot CH_2\cdot CH=$(three), $=CH\cdot(CH_2)_2\cdot CH=$(two), $=CH\cdot(CH_2)_2\cdot COOH$.
(2) $CH_3\cdot CH=$, $=CH\cdot CH_2\cdot CH=$(four), $=CH\cdot(CH_2)_2\cdot CH=$, $=CH\cdot(CH_2)_2\cdot COOH$.

No evidence was brought out in these experiments regarding the respective positions of the groups $=CH\cdot CH_2\cdot CH=$ and $=CH\cdot(CH_2)_2\cdot CH=$. If, however, the group $=CH\cdot(CH_2)_2\cdot CH=$ is assumed to lie near the carboxyl group side of docosahexenoic acid, as is the case with previously studied moroctic,(4) eicosatetraenoic(5) and clupanodonic acids,(6) the constitution of docosahexenoic acid is to be expressed by either one of the following formulae:

$$CH_2=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot(CH_2)_2\cdot CH= \\ CH\cdot(CH_2)_2\cdot CH=CH\cdot(CH_2)_2\cdot COOH \quad \text{(I)}$$

$$CH_3\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH=CH\cdot CH_2\cdot CH= \\ CH\cdot(CH_2)_2\cdot CH=CH\cdot(CH_2)_2\cdot COOH \quad \text{(II)}$$

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(2) This Bulletin, **10** (1935), 441.

(3) Propyl aldehyde must have been formed, though in a small amount, by the ozonolysis of the amyl ester, since the ester was contaminated with amyl clupanodonate.

(4) This Bulletin, **10** (1935), 232.

(5) This Bulletin, **10** (1935), 296.

(6) This Bulletin, **10** (1935), 441.

The yield of C_4-compounds obtained by the ozonolysis could not be determined accurately, but it seemed to indicate the presence of more than one of the group $=CH\cdot(CH_2)_2\cdot CH=$ in amyl docosahexenoate. Accordingly the formula (I) seems most probable. Further experiments will be done when pure docosahexenoic acid is obtained in sufficient quantity.

Experimental.

Docosahexenoic acid used in these experiments is the same sample which was obtained in the previous study.(7) It had d_4^{15} 0.9521, n_D^{15} 1.5129, neutr. value 170.1 (calc. 170.9), iodine value by the Wijs method 456.0 (calc. 464.0) and gave 177% of an ether insoluble bromide having Br-content 74.00% (calc. for $C_{22}H_{32}O_2Br_{12}$: 74.50%). Assuming that this sample is contaminated exclusively with clupanodonic acid, the purity of docosahexenoic acid is calculated to be 89.97% from its iodine value. The free acid was converted into its amyl ester by heating with an equal amount of amyl alcohol containing 2.5% of hydrogen chloride on the water-bath under a reflux condenser for one hour. Five g. of the amyl ester thus obtained was dissolved in 50 c.c. of chloroform, cooled with ice-salt, and a current of ozonised oxygen was passed into the solution until it became saturated with ozone. On distilling chloroform from the solution under diminished pressure, there was obtained 8.9 g. of ozonide as an orange yellow syrup. Chloroform was, however, not thoroughly removed from the ozonide, since there was a danger of explosive decomposition on undue heating. Water (50 c.c.) was added to the ozonide, and the liquid was heated on the water-bath in a current of hydrogen. The flask was attached by a delivery tube to other three flasks (**a**, **b**, and **c**) which were connected in succession; the first (**a**) was filled with ice cold water, the second (**b**) and the third (**c**) with approximately 1/3 N barium hydroxide solution. The volatile decomposition products (**A**) carried over with hydrogen were passed into the above three flasks where they were caught by absorption. The decomposition products remained in the initial flask dissolved partly in water, and the remainder separated as oily substances (**C**) under aqueous solution (**B**).

1. **Volatile Decomposition 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. On addition of *p*-nitrophenylhydrazine and hydrochloric acid, there was formed a precipitate of *p*-nitrophenylhydrazone which on recrystallisation from 50% alcohol gave yellow needles with m.p. 125–126° which was not lowered when the substance was admixed with a pure specimen of *p*-nitrophenylhydrazone of acetaldehyde (m.p. 128°) in various proportions.

The barium hydroxide solution in the flasks (**b**) and (**c**) were found to contain a precipitate of barium carbonate, indicating that carbon dioxide was formed by the ozonolysis. The quantity of carbon dioxide calculated from the quantity of barium carbonate was found to be 1.26 g. or 25.2% of the amyl ester used for ozonolysis.

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(7) This Bulletin, **10** (1935), 433.

This is higher than the maximum yield (22.1%) of carbon dioxide obtainable on assumption that amyl docosahexenoate has two of the group $=CH{\cdot}CH_2{\cdot}CH=$, and that the products of ozonolysis derived from that group undergoes secondary decomposition with the formation of carbon dioxide to a quantitative extent. Consequently amyl docosahexenoate must have at least three of the group $=CH{\cdot}CH_2{\cdot}CH=$.

2. **Aqueous Solution (B).** This was extracted with a large quantity of ether. On removal of ether from the ethereal extract, there remained 3.6 g. of a reddish orange liquid, which was then treated with petroleum ether and was separated into petroleum ether solution and petroleum ether insoluble portion. On distilling off petroleum ether from the solution, the distillate (petroleum ether) had a smell of acetic acid. The residue was then heated in an oil-bath, yielding 0.6 g. of a colourless distillate below 150° of the bath-temperature. It had a smell of acetic acid, and the silver salt prepared from it was proved to be silver acetate by analysis (Found: Ag, 64.48. Calc. for $C_2H_3O_2Ag$, 64.64%).

The petroleum ether insoluble portion (1.9 g.) consisted of a mixture of reddish orange liquid and crystalline solid, and showed aldehydic character. Oxidation with an alkaline solution of potassium permanganate followed by acidification yielded a crystalline product which, after being washed with a little cold ether, showed neutr. value 947.6 (calc. for succinic acid $C_4H_6O_4$: 950.6) and m.p. 179–180°. Recrystallisation from ethyl acetate yielded pure succinic acid with m.p. and mixed m.p. 182–183°. The petroleum ether insoluble portion was considered to consist almost exclusively of C_4-compounds (succinic acid and the corresponding aldehyde), and the yield was 1.9 g. or 38% of the amyl ester used for ozonolysis. This is considerably higher than the maximum yield of C_4-compounds (as succinic acid) 27.1% which is obtainable on condition that amyl docosahexenoate has only one of the group $=CH{\cdot}(CH_2)_2{\cdot}CH=$.

3. **Oily Substances (C).** Yield 1.9 g. These substances had acid and aldehydic characters. Oxidation with alkaline permanganate followed by acidification yielded an acid ester which showed neutr. value 297.1 and saponification value 594.8, and was considered to be amyl hydrogen succinate $C_9H_{16}O_4$ (neutr. value 298.2 and saponif. value 596.5). The free acid liberated from its acid ester in the usual way yielded succinic acid after recrystallisation from ethyl acetate; neutr. value 946.9 and m.p. 182–183°. From these results it is seen that the oily substances consist chiefly of amyl hydrogen succinate and the corresponding aldehyde.

Summary.

1. A concentrated fraction of docosahexenoic acid was converted into the amyl ester, and the latter was subjected to ozonolysis. Among the products of ozonolysis were found: acetaldehyde, acetic acid, carbon dioxide, succinic acid and amyl hydrogen succinate. The presence of the aldehydes corresponding to succinic acid and amyl hydrogen succinate was also indicated. Of these compounds, carbon dioxide together with acetaldehyde and acetic acid is attributable to the secondary decomposition of the products derived from the group $=CH{\cdot}CH_2{\cdot}CH=$. The yield of carbon dioxide indicated the presence of at least three of the group

$=CH\cdot CH_2\cdot CH=$ in amyl docosahexenoate. Consequently docosahexenoic acid has the following groups: $=CH\cdot CH_2\cdot CH=$, $=CH\cdot(CH_2)_2\cdot CH=$, and $=CH\cdot(CH_2)_2\cdot COOH$, of which it has at least three of the group $=CH\cdot CH_2\cdot CH=$. The yield of C_4-compounds (succinic acid and the corresponding aldehyde) obtained by the ozonolysis gave an indication of the presence of more than one of the group $=CH\cdot(CH_2)_2\cdot CH=$ in docosahexenoic acid.

2. Although the group attached to the CH_3-side and the respective positions of the groups $=CH\cdot CH_2\cdot CH=$ and $=CH\cdot(CH_2)_2\cdot CH=$ were left undetermined in these experiments, the most probable structure of docosahexenoic acid was derived from the results obtained above.

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Correction to the 7th paper on the Highly Unsaturated Acids in Sardine Oil:

Read n_D^{20} 1.5014 *for* n_D^{20} 1.4934 *in line 16 from bottom on page 439 of this volume.*