THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL. VI. THE CONSTITUTION OF EICOSAPENTENOIC ACIDS $C_{20}H_{30}O_2$.

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On examining the highly unsaturated C_{20}-acids in sardine oil, we separated an eicosatetraenoic acid and a concentrated fraction of eicosapentenoic acid as described in the 4th report of this series.(1) In the 5th report,(2) the constitution of the eicosatetraenoic acid was established as $\Delta^{4:5,\ 8:9,\ 12:13,\ 16:17}$-acid. The present paper deals with the determination of the constitution of the eicosapentenoic acid by examining the products of the ozonolysis of the amyl ester prepared from the concentrated fraction of eicosapentenoic acid which had been obtained in the previous study. The concentrated fraction of eicosapentenoic acid used for this experiment, however, contained a considerable amount of eicosatetraenoic acid. It was, therefore, expected from the beginning that the result of the present experiments would not bring sufficient data to establish the constitution of the eicosapentenoic acid. A further experiment will be carried out when the pure eicosapentenoic acid is separated in sufficient quantity.

Among the products obtained by the ozonolysis of the concentrated fraction of amyl eicosapentenoate were found: succinic acid $HOOC\cdot(CH_2)_2\cdot COOH$, amyl ester of succinic semi-aldehyde $HOC\cdot(CH_2)_2\cdot COOC_5H_{11}$, amyl hydrogen succinate $HOOC\cdot(CH_2)_2\cdot COOC_5H_{11}$, carbon dioxide CO_2, acetic acid $CH_3\cdot COOH$ and acetaldehyde $CH_3\cdot COH$. A higher aldehyde in addition to acetaldehyde seemed to be present. Of these compounds a portion of succinic acid, amyl hydrogen succinate and the corresponding semi-aldehyde must have been derived from amyl eicosatetraenote which contaminated the amyl ester fraction used for the ozonolysis, but it was ascertained from the yields of these compounds that not only amyl eicosatetraenoate but also amyl eicosapentenoate itself yielded these compounds, and accordingly eicosapentenoic acid has the groups $=CH\cdot(CH_2)_2\cdot CH=$ and $=CH\cdot(CH_2)_2\cdot COOH$. Carbon dioxide was formed obviously by the secondary decomposition of the products of ozonolysis

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(1) This Bulletin, **10** (1935), 241.

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

derived from the group $=CH\cdot CH_2\cdot CH=$, and since amyl eicosatetraenoate does not contain the group $=CH\cdot CH_2\cdot CH=$, carbon dioxide obtained in the present experiment must have been derived exclusively from amyl eicosapentenoate; a comparatively large yield of carbon dioxide indicates that amyl eicosapentenoate has more than one of the group $=CH\cdot CH_2\cdot CH=$. Although acetic acid and acetaldehyde are formed together with carbon dioxide by the secondary decomposition of the products of ozonolysis derived from the group $=CH\cdot CH_2\cdot CH=$, it should be taken into consideration that when amyl eicosapentenoate has the group $CH_3\cdot CH=$, it yields acetic acid and acetaldehyde on ozonolysis. A higher aldehyde which seemed to be present in addition to acetaldehyde could not be identified, but it was supposed to be butyric aldehyde derived from the contaminating eicosatetraenoate. From these results, eicosapentenoic acid is shown to contain the following groups: $=CH\cdot(CH_2)_2\cdot COOH$, $=CH\cdot(CH_2)_2\cdot CH=$ and $=CH\cdot CH_2\cdot CH=$, of which eicosapentenoic acid contains more than one of the group $=CH\cdot CH_2\cdot CH=$. The group at the CH_3-side was left undetermined, and consequently the constitution of eicosapentenoic acid could not be definitely established. If, however, eicosapentenoic acid is assumed to have ethylenic linkings at 4:5-, 8:9- and 12:13-positions as in the case of moroctic acid ($\Delta^{4:5,\ 8:9,\ 12:13,\ 15:16}$) and eicosatetraenoic acid ($\Delta^{4:5,\ 8:9,\ 12:13,\ 16:17}$), we can ascribe from the results of the present experiment the following constitution ($\Delta^{4:5,\ 8:9,\ 12:13,\ 15:16,\ 18:19}$) to eicosapentenoic acid.

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

Experimental.

The concentrated fraction of eicosapentenoic acid used for this experiment was the same sample as described in the 4th report, and had d_4^{15} 0.9399, n_D^{15} 1.5109, neutralisation value 185.1, iodine value by the Wijs method 392.7. As is seen from the iodine value, this fraction still contained a considerable amount of eicosatetraenoic acid (iodine value 333.7) in addition to eicosapentenoic acid (iodine value 419.9). Assuming that this fraction consists exclusively of the two acids, the percentage of eicosapentenoic acid is found to be 68.44%. The above fraction was refluxed with an equal amount of amyl alcohol containing 2.5% of hydrogen chloride for one hour, and the resulting amyl ester was freed from unchanged free acids and the excess of amyl alcohol. The amyl ester (5.5 g.) thus obtained was dissolved in chloroform (50 c.c.), the solution was cooled with ice-salt, and a current of ozonised oxygen was passed through the solution until it became saturated. On distilling off chloroform under reduced pressure, the ozonide was obtained as an orange yellow syrup. As the ozonide was extremely unstable, the last traces of chloroform were not removed. Water (50 c.c.) was added to the ozonide in a flask, and the liquid was heated on the water-bath for 30 minutes, a current of hydrogen being passed into the flask. The volatile substances (**A**) carried over with hydrogen were collected in three flasks (**a**, **b** and **c**) as in the case of previous experiments of ozonolysis; the first (**a**) was filled with 50 c.c. of water under cooling, the second (**b**) and the third (**c**) with 200 c.c. of approximately 1/3N solution of barium hydroxide. The products remained in the initial flask were separated into aqueous solution (**B**) and oily substances (**C**).

1. **Volatile Substances (A).** These substances were collected in the three flasks (**a**, **b** and **c**). 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 in the solution. The *p*-nitrophenylhydrazone prepared from this solution showed m.p. 118–119° after recrystallisation from 50% alcohol (Found: N, 22.61. Calc. for $C_8H_9O_2N_3$: N, 23.46%). The N-content is lower than the calculated value for *p*-nitrophenylhydrazone of acetaldehyde, indicating the presence of higher aldehyde besides acetaldehyde. Although this higher aldehyde was not identified, it should be noted here that the aqueous solution in the flask (**a**) should contain a higher aldehyde such as butyric aldehyde besides acetaldehyde since the amyl ester used for ozonolysis was contaminated with amyl eicosatetraenoate which on ozonolysis yielded butyric aldehyde. The aqueous solution in the flask (**a**) showed also acid reaction and produced a deep red colouration on addition of 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 considerable amount of a precipitate of barium carbonate which was also found in a minor amount in the barium hydroxide solution in the flask (**c**), and consequently carbon dioxide must have been formed by the ozonolysis. The formation of carbon dioxide is to be attributed to the secondary decomposition of the products of ozonolysis derived from the group $=CH\cdot CH_2\cdot CH=$, and since amyl eicosatetraenoate contaminated the amyl ester used for ozonolysis has no such group, amyl eicosapentenoate must have that group. Calculating from the iodine value 392.7, the total quantity of amyl eicosapentenoate contained in 5.5 g. of the amyl ester used for ozonolysis was found to be 3.77 g. Calculating from the quantity of the precipitate of barium carbonate, on the other hand, the quantity of carbon dioxide formed by the ozonolysis was found to be 0.69 g. or 18.3% of the quantity (3.77 g.) of amyl eicosapentenoate. This is considerably higher than the calculated yield 11.8% which is obtainable on assumption that amyl eicosapentenoate has only one of the group $=CH\cdot CH_2\cdot CH=$ and that the secondary decomposition of the products of ozonolysis derived from that group takes place to a quantitative extent. Consequently amyl eicosapentenoate must contain more than one of the group $=CH\cdot CH_2\cdot CH=$.

2. **Aqueous Solution (B).** The substances dissolved in aqueous solution (**B**) were extracted with ether (500 c.c.), and on distilling off ether from the ethereal solution, there was obtained 4 g. of a reddish orange liquid which was treated with 50 c.c. of petroleum ether in order to effect a separation into petroleum ether insoluble portion and petroleum ether solution.

Petroleum ether insoluble portion (3 g.) was oxidised with alkaline permanganate, and the product, after being acidified and washed with a little ether, was recrystallised from ethyl acetate, yielding impure succinic acid; neutralisation value 945.9 (calc. for $C_4H_6O_4$: 950.6), m.p. 179–180°. Hence, this portion is believed to consist of succinic acid and the corresponding aldehyde. Although these C_4-compounds may partly be derived from amyl eicosatetraenoate contaminating the amyl ester used for ozonolysis, the yield of this portion indicates that these compounds have been derived also from amyl eicosapentenoate. On distilling off the petroleum ether solution, the distillate of petroleum ether showed acid reaction, and the residue had a smell of acetic acid.

3. **Oily Substances (C).** This portion (2.1 g.) was neutralised with sodium carbonate solution, and the neutral substances were extracted with ether.

The acidic substances, obtained from the neutralised solution on acidification, had neutralisation value 295.3 and saponification value 594.1, indicating that they consisted principally of amyl hydrogen succinate $C_9H_{16}O_4$ (neutralisation value 298.2, saponification value 596.5). The free acid obtained from this mono-amyl ester yielded, on recrystallisation from ethyl acetate, succinic acid; neutralisation value 947.1, m.p. 182–183°. Oxidation of the neutral substances with alkaline permanganate gave an acid ester having neutralisation value 295.1 and saponification value 592.1. The free acid obtained from the acid ester yielded impure succinic acid melting at 179–180° after recrystallisation from ethyl acetate. From these results, the oily substances (**C**) were found to consist mainly of amyl hydrogen succinate and the corresponding semi-aldehyde.

Summary.

A concentrated fraction of eicosapentenoic acid containing an appreciable amount of eicosatetraenoic acid was converted into amyl ester and the latter was subjected to ozonolysis. Among the products of ozonolysis were found: succinic acid, amyl hydrogen succinate, amyl ester of succinic semi-aldehyde, carbon dioxide, acetic acid, acetaldehyde and a higher aldehyde (probably butyric aldehyde). The yield of these compounds and also the constitution of eicosatetraenoic acid ($\Delta^{4:5,\ 8:9,\ 12:13,\ 16:17}$) being taken into consideration, it was concluded that the eicosapentenoic acid had 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 contained more than one of the group $=CH\cdot CH_2\cdot CH=$. As the group at the CH_3-side was left undetermined in these experiments, the constitution of the eicosapentenoic acid was not established with certainty. If, however, it is assumed that there are ethylenic linkings in 4:5-, 8:9- and 12:13-positions like in the eicosatetraenoic acid, the constitution must be $\Delta^{4:5.\ 8:9,\ 12:13,\ 15:16,\ 18:19}$-eicosapentenoic acid as expressed by the following formula:

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

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