THE HIGHLY UNSATURATED ACIDS IN SARDINE OIL. XIII. OXIDATION OF METHYL CLUPANODONATE WITH POTASSIUM PERMANGANATE IN ACETONE SOLUTION.

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The constitution of clupanodonic acid, which forms an important constituent of the highly unsaturated acids in sardine oil, was dealt with in the 8th report(1) of this series. Amyl clupanodonate was subjected to ozonolysis, and propionic aldehyde, acetaldehyde, carbon dioxide, succinic acid and amyl hydrogen succinate were identified among the products of ozonolysis. Also the presence of lower acids, which were deemed to consist of propionic and acetic acids, was indicated. Of these compounds propionic aldehyde and propionic acid are derived from the group $CH_3{\cdot}CH_2{\cdot}CH{=}$, while acetaldehyde, acetic acid together with carbon dioxide are formed by a further degradation of the products of ozonolysis derived from the group $={CH}{\cdot}CH_2{\cdot}CH{=}$. Succinic acid and amyl hydrogen succinate are evidently derived from the groups $={CH}{\cdot}(CH_2)_2{\cdot}CH{=}$ and $={CH}{\cdot}(CH_2)_2{\cdot}COOC_5H_{11}$ respectively. Hence the groups jointed by five ethylenic linkings in clupanodonic acid were found to be $CH_3{\cdot}CH_2{\cdot}CH{=}$, $={CH}{\cdot}CH_2{\cdot}CH{=}$, three of $={CH}{\cdot}(CH_2)_2{\cdot}CH{=}$ and $={CH}{\cdot}(CH_2)_2{\cdot}COOH$. For the determination of the positions of the respective groups, we made use of the fact that the addition of bromine to the ethylenic linkings in clupanodonic acid proceeds selectively, and prepared dibromo-, tetrabromo- and hexabromo-derivatives of clupanodonic acid by the selective addition of bromine to the ethylenic linkings which lie more distant from the carboxyl group. The products of ozonolysis of these bromo-derivatives were examined, and the following formula of clupanodonic acid was established.

$$\begin{aligned}&CH_3{\cdot}CH_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}CH{=}CH{\cdot}CH_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}\\&\qquad CH{=}CH{\cdot}(CH_2)_2{\cdot}CH{=}CH{\cdot}(CH_2)_2{\cdot}COOH.\end{aligned}$$

Before our experiment, Inoue and Sahashi(2) oxidised the methyl ester of clupanodonic acid with potassium permanganate in acetone solution, and found

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

butyric, pimelic, succinic, adipic and oxalic acids among the oxidation products. Adipic and oxalic acids were thought by these authors to be formed by the secondary decomposition of pimelic and malonic acids, and consequently the oxidation products primarily derived from the groups jointed by unsaturated linkings in clupanodonic acid were found to be butyric, pimelic, succinic (2 mols), and malonic acids. For the determination of the positions of the respective groups, these authors subjected clupanodonic acid to partial hydrogenation and separated the product into the fractions of different degrees of saturation. The positions of the unsaturated linkings in each fraction were examined by the oxidation with potassium permanganate, and the following formula having one triple linking and three double linkings was proposed for clupanodonic acid.

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

While we used the ozonide method for the determination of the constitution of clupanodonic acid, Inoue and Sahashi used the permanganate method for the same purpose, but the difference of the oxidation method can not explain well the marked discrepancies between the results of our previous experiment and those obtained by Inoue and Sahashi. Leaving the positions of the respective groups jointed by unsaturated linkings undiscussed, it can hardly be accepted that the oxidation products obtained in our experiment on the amyl ester of clupanodonic acid by the ozonide method and those obtained by Inoue and Sahashi from the methyl ester of clupanodonic acid by the permanganate method resulted from the same single compound. It should, however, be noted that while the specimen of clupanodonic acid used for the determination of the constitution in our experiment was freed from C_{22}-acids of different degrees of unsaturation as far as possible and possessed high purity, the purities of specimens of clupanodonic acid which had been separated by several authors before our experiment are doubtful from the contamination of acids of different degrees of unsaturation. But such difference in the purity of clupanodonic acid alone can not explain the discrepancies between the results of our experiment and those obtained by Inoue and Sahashi. The following descriptions are the results of our experiment on the oxidation of methyl clupanodonate with potassium permanganate in acetone solution.

Among the products of oxidation of methyl clupanodonate with potassium permanganate in acetone solution, propionic acid, acetic acid, succinic acid and methyl hydrogen succinate were identified. Acetic acid is believed to be formed by the secondary decomposition of malonic acid, and consequently the groups jointed by ethylenic linkings in clupanodonic acid were found to be $CH_3{\cdot}CH_2{\cdot}CH=$, $=CH{\cdot}(CH_2)_2{\cdot}CH=$, $=CH{\cdot}CH_2{\cdot}CH=$ and $=CH{\cdot}(CH_2)_2{\cdot}COOH$, of which three of the group $=CH{\cdot}(CH_2)_2{\cdot}CH=$ are present since clupanodonic acid has 22 carbon atoms in total. These results agree fully with those obtained by the ozonolysis of amyl clupanodonate in the previous experiment. Whilst the finding of the group $CH_3{\cdot}(CH_2)_2{\cdot}CH=$ by Inoue and Sahashi at the terminal opposite to the carboxyl group is based on the detection of butyric acid among the oxidation products, we could not find butyric acid, but we separated propionic acid which was identified as its *p*-bromophenacyl ester in pure state. Neither adipic acid nor pimelic acid could be found among the oxidation products in our experiment. In short, the oxidation products obtained in our experiment on methyl clupanodonate by means of potassium permanganate differ from those reported by Inoue and Sahashi, and the results of our experiment agree fully with those of the previous experiment of the ozonolysis of amyl clupanodonate. It is quite certain that clupanodonic acid has no triple linking but it has five ethylenic linkings. The structure is $\Delta^{4:5,\,8:9,\,12:13,\,15:16,\,19:20}$-docosapentenoic acid as has been established by the previous experiment.

Experimental.

1. Oxidation of Methyl Clupanodonate and Separation of Oxidation Products. Methyl clupanodonate used in the present experiment was the same as described in the 7th report⁽³⁾ of this series, and had d_4^{15} 0.9240, n_D^{15} 1.4955, saponif. value 163.0 (calc. 163.0) and iodine value (Wijs) 368.0 (calc. 368.6). Amyl clupanodonate and free clupanodonic acid used for the experiment of ozonolysis described in the 8th report⁽⁴⁾ of this series were prepared from the same specimen of methyl clupanodonate.

Ten grams of methyl clupanodonate was dissolved in 200 c.c. of acetone, and 150 g. of potassium permanganate was added in small portions. In order to avoid a violent heat evolution, the solution was cooled at first and then potassium permanganate was added. After the heat evolution has ceased, the solution was heated on the water bath under a reflux condenser. Before the addition of a further quantity of potassium permanganate the solution was cooled, and then potassium permanganate was added. After the heat evolution has ceased, the solution was again heated. Repeating these operations the entire quantity of potassium permanganate was added in the course of 5 hours. The solution was refluxed for 5 hours more, and then the oxidation products were separated into several fractions as shown in Table 1.

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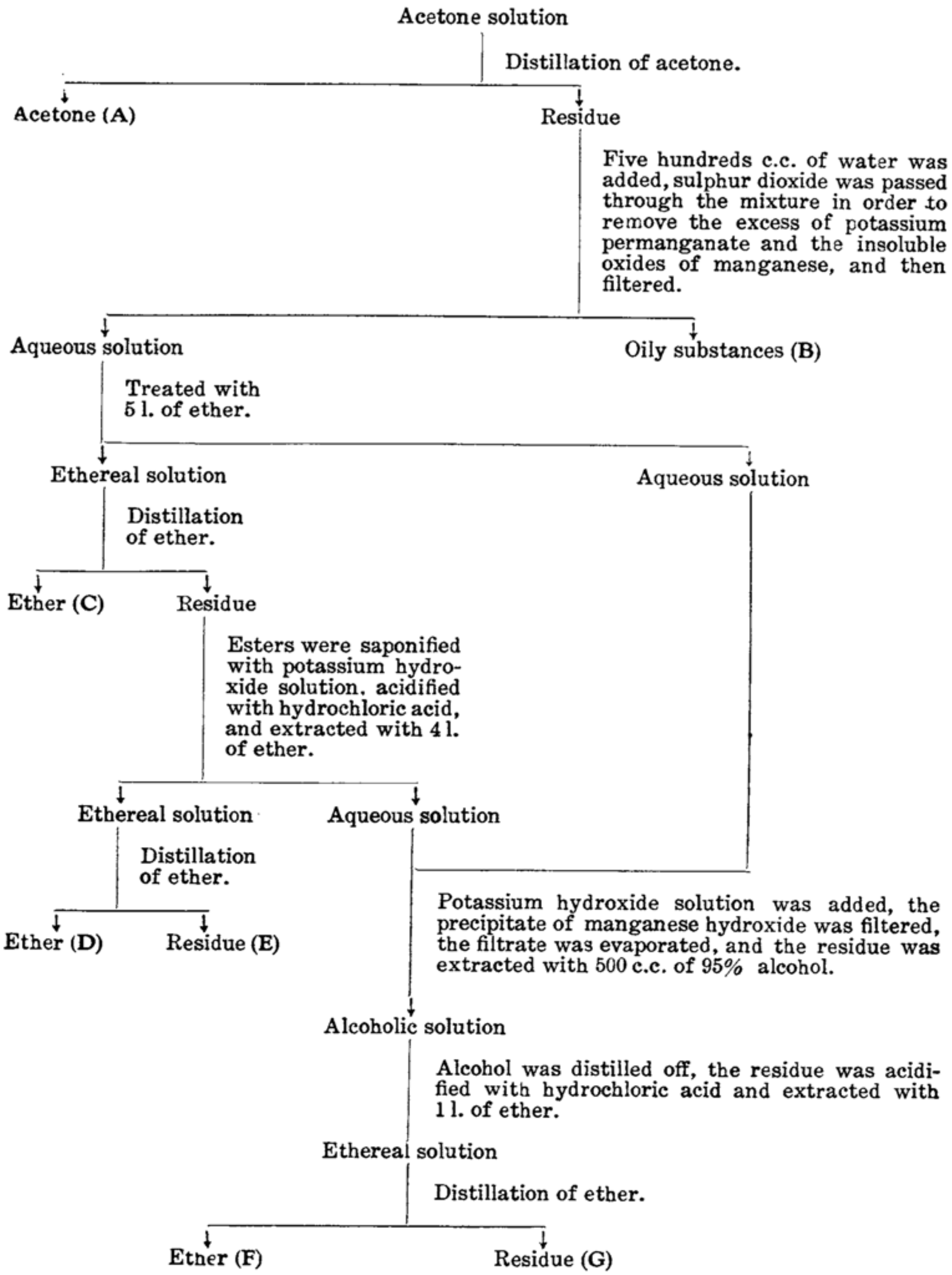
(3) This Bulletin, **10** (1935), 433.

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

Table 1.



Five hundreds c.c. of water was added, sulphur dioxide was passed through the mixture in order to remove the excess of potassium permanganate and the insoluble oxides of manganese, and then filtered.

Esters were saponified with potassium hydroxide solution, acidified with hydrochloric acid, and extracted with 4 l. of ether.

Potassium hydroxide solution was added, the precipitate of manganese hydroxide was filtered, the filtrate was evaporated, and the residue was extracted with 500 c.c. of 95% alcohol.

Alcohol was distilled off, the residue was acidified with hydrochloric acid and extracted with 1 l. of ether.

2. **Oxidation Products Distilled off with the Solvents.** The acetone (A) and the ether (C, D, F), which were recovered in the separative operations shown in Table 1, showed acidic reaction. The acetone (A) was neutralised with potassium hydroxide, acetone was distilled off, and there remained the potassium salts. The ether (C, D, F) was shaken with potassium hydroxide solution by which the acidic substances were converted into the potassium salts and separated from the ethereal solution. The aqueous solution of potassium salts was evaporated to dryness and there remained the potassium salts as residue. The potassium salts thus obtained were united and treated with warm 95% alcohol in which potassium sulphite remained insoluble. The alcoholic solution was separated and evaporated. The residue was acidulated with dilute sulphuric acid and distilled. The distillate still contained sulphur dioxide. For the removal of last traces of sulphur dioxide, potassium permanganate was added to the distillate, and the solution was redistilled. The yield of final distillate was 43 g., and it had acid value 9.6, from which the total amount of acids contained in the distillate was calculated to be 0.44 g. as acetic acid. Thirty grams of distillate was nearly neutralised with N/10 alcoholic potash, and after addition of 1.4 g. of *p*-bromophenacyl bromide the solution was refluxed for 2 hours. A large amount of water was then added, and the separated solid was recrystallised from 60% alcohol. The crystals obtained melted at 84.5–85° both alone and after admixture with a pure specimen of *p*-bromophenacyl ester of acetic acid.

3. **Oxidation Products Dissolved in Water.** The residues (E) and (G) in Table 1 consisted of a mixture of crystalline solid and liquid. These were united and treated with petroleum ether (b. p. below 50°). The petroleum ether solution and the insoluble portion were separated.

(i) *Petroleum ether solution.* The solution was first heated on the water bath until the bulk of petroleum ether distilled over. It was then heated in an oil bath, and the distillate, which distilled over below 100° and was contaminated with a large proportion of petroleum ether, was collected and united with petroleum ether obtained before. The yield of distillate thus obtained was 154 g. It had acid value 4.3, from which the acidic substances contained in the distillate were calculated to be 0.71 g. as acetic acid. The *p*-bromophenacyl ester was prepared from the acidic substances contained in the distillate, and it was recrystallised from 60% alcohol yielding crystals which melted at 82–83° and showed no depression of melting point when admixed with a pure specimen of *p*-bromophenacyl ester of acetic acid (m. p. 84.5°) in various proportions. The portion which did not distil below 100° amounted to 1.9 g. It yielded two fractions on further heating: (1) b.p. 100–130°, 0.25 g.; (2) b. p. 130–140°, 0.8 g. Above 140° practically no distillate was obtained, and when the temperature of the oil bath reached 200° the residue began to show the indications of decomposition. The fraction (1) consisted mainly of propionic acid, and had n_D^{20} 1.3871 and neutr. value 763.2 (calc. 757.7). The *p*-bromophenacyl ester, when recrystallised from 70% alcohol, melted at 62.5–63° both alone and after admixture with a pure specimen of *p*-bromophenacyl ester of propionic acid. The final residue from distillation was a dark brownish viscous oil which was considered to be intermediate products which escaped a complete oxidation. It was subjected to a further oxidation by means of an alkaline solution of potassium permanganate, and the products were acidified and taken up with ether. On removal of ether from ethereal solution, there remained a crystalline solid (0.1 g.) which appeared to be impure succinic acid from its melting point 178–179°.

(ii) *The portion insoluble in petroleum ether.* This portion (7.5 g.) consisted of a mixture of reddish orange liquid and crystalline solid. It was washed with cold ether for the removal of liquid portion, and the crystalline solid (a) was separated. The liquid portion obtained from the ethereal washing appeared to contain the substances which escaped a complete oxidation. It was subjected to a further oxidation with an alkaline solution of potassium permanganate. After acidification the products were taken up with ether, and on removal of ether there was obtained crystalline solid (b) which was still contaminated with a small proportion of oily substances. The crystalline solids (a) and (b) were united and recrystallised from ethyl acetate, yielding 1st crop of neutr. value 950.1 and m.p. 181–182° and 2nd crop of neutr. value 950.0 and m.p. 180–181°. Each crop showed no depression of melting point when admixed with a pure specimen of succinic acid (neutr. value calc. 950.6, m.p. 182–183°).

4. **Oily Substances.** Yield 3.6 g. These substances (B) contained some neutral substances which escaped oxidation. For the separation of acidic substances, the mixture was dissolved in ether and shaken with potassium hydroxide solution. The acidic substances were converted into the potassium salts and separated from ethereal solution. After the acidification of the aqueous solution of potassium salts with hydrochloric acid, the acidic substances liberated were taken up with ether. On distillation of ether, there were obtained 1.9 g. of acidic substances which showed neutr. value 432.1 and saponif. value 835.1 which are close to the calculated values for methyl hydrogen succinate (neutr. value 424.8, saponif. value 849.7). The free acid prepared from this acid ester by saponification followed by acidification melted at 179–180° after being washed with a little cold ether. The melting point was not lowered when the substance was admixed with succinic acid in various proportions.

Summary.

Methyl clupanodonate has been oxidised with potassium permanganate in acetone solution. Among the oxidation products propionic acid, acetic acid, succinic acid and methyl hydrogen succinate have been identified. Of these compounds acetic acid is believed to be formed by the secondary decomposition of malonic acid. Accordingly the presence of above-mentioned oxidation products gives an indication of the presence of the following groups in clupanodonic acid jointed by ethylenic linkings thus: $CH_3{\cdot}CH_2{\cdot}CH{=}$, $={CH}{\cdot}CH_2CH{=}$, three of $={CH}{\cdot}(CH_2)_2{\cdot}CH{=}$, and $={CH}{\cdot}(CH_2)_2{\cdot}COOH$. These results agree fully with those obtained in a previous experiment of the ozonolysis of amyl clupanodonate.

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