

# VOLATILE COMPOUNDS FORMED BY DECOMPOSITION OF THE PEROXIDES OF HIGHLY UNSATURATED ACIDS AND THEIR METHYL ESTERS

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A previous study by Toyama and Matsumoto<sup>1)</sup> indicated the presence of formic, acetic, butyric and heptic (or caproic) acids, acetaldehyde, butyraldehyde and *n*-heptanal in the volatile compounds formed by passing air into the methyl esters of highly unsaturated acids, prepared from sardine oil, at 45°-55°C. Later Toyama and Suzuki<sup>2)</sup> oxidized the highly unsaturated methyl esters at 0°-25°C by shaking them in an atmosphere of oxygen to give the oxidized methyl esters of high peroxide values of which the highest value was 2,220. They found formic, acetic and propionic acids, butyraldehyde together with its lower homologues, *n*-hexanal and three unsaturated aldehydes—crotonaldehyde, 2-pentenal and 2-hexenal—in the volatile substances formed by heating the oxidized methyl esters at 90°-100°C in a current of nitrogen. In the present study, the highly unsaturated acids and their methyl esters were oxidized at room temperature by the shaking of the materials in an atmosphere of air or oxygen to give oxidized materials of relatively low peroxide values, mostly below 100 with a few exceptions. The oxidized materials were then heated at a temperature above 100°C in a current of nitrogen, and the volatile compounds formed were examined. In another series of experiments in this study, the highly unsaturated acids and methyl esters were spread on absorbent cotton, and the materials were kept in contact with air in our laboratory room in order to oxidize the highly unsaturated materials. After the materials had been oxidized to some extent, the materials together with absorbent cotton were heated at 60°-70°C in a current of nitrogen, and the volatile compounds formed were examined. The highest peroxide value of the oxidized materials in this series of experiments was 413.

## 1. Oxidation of the Highly Unsaturated Acids and Their Methyl Esters and Decomposition of the Peroxides

The highly unsaturated materials used in this study are two samples (I and II) of highly unsaturated acids and two samples (III and IV) of highly unsaturated methyl esters. The sample I (neutralization value 183.3 and iodine value 301.5) and the sample II (neutralization value 178.5 and iodine value 318.5) were prepared from sardine oil by means of the sodium salt acetone method. The sample III (saponification value 183.2 and iodine value 295.9) and the sample IV (saponification value 177.9 and iodine value 359.6) were prepared from the fatty

acids of sardine oil by separating a fraction rich in highly unsaturated acids using urea followed by conversion of this fraction to its methyl esters and subsequent distillation of the methyl esters under vacuum.

The oxidation-decomposition procedures for each sample were performed as follows. With the samples I and IV, each sample was placed in a flask which was connected through a rubber tube to a holder containing air or oxygen under nearly atmospheric pressure. The flask was set on a shaking apparatus. After shaking for some period, the flask containing oxidized samples was transferred to an oil bath in order to decompose peroxides in the oxidized sample. A current of nitrogen was steadily passed into the flask during heating. After the completion of decomposition, the flask with its content was again set on the shaking apparatus and the material in the flask was oxidized as before. Thus the initial sample was repeatedly subjected to oxidation-decomposition procedures. With the samples II and III, each sample was spread on one-tenth its weight of absorbent cotton, and the mixture was allowed to contact with air in our laboratory room. When the sample was oxidized to some extent, the mixture of oxidized sample and absorbent cotton was put into a glass tube. The wall of this tube was heated using a water bath, and a current of nitrogen was steadily passed through the tube. The peroxides formed in the oxidized sample was thus decomposed. After the completion of decomposition, the mixture of sample and absorbent cotton was taken off from the tube and allowed to contact with air for some period. The oxidation-decomposition cycle was thus repeated for each sample. Detailed conditions of oxidation-decomposition procedures for each sample are given in Table 1.

TABLE 1. Oxidation of Samples and Decomposition of Peroxides

Exp. No.	Sample	Method of oxidation	Number of repetition of oxidation-decomposition	Oxidation			Decomposition	
				Room temperature (°C)	Period of each oxidation procedure (hr.)	Peroxide value after each oxidation procedure (hr.)	Temperature (°C)	Period of each decomposition procedure (hr.)
1	I	Shaking in an atmosphere of air	32	15-23	20- 60	18- 80	120-125	1.5-4
2	IV	"	9	10-25	68-166	31- 90	100	2 -4
3	IV	Shaking in an atmosphere of oxygen	2	5-15	77-244	135-172	100	1.5-2
4	II	Spread on absorbent cotton, and allowed to contact with air	6	15-25	17- 43.5	54-217	60- 70	4 -7
5	II	"	5	5-15	25- 69	19-330	60- 70	2 -5.5
6	III	"	5	5-15	24- 68.5	43-413	60- 70	2 -5

Notes: Peroxide values (milliequivalent/kg.) were determined by the method of Wheeler. Peroxide values after each decomposition procedure were mostly below 1 in the case of Exp. 1, but were larger in the case of Exps. 2-6. Especially in the case of Exps. 4-6, the decomposition of peroxides was incomplete due to a relatively low temperature, and the peroxide values after the decomposition procedure were sometimes remarkably high.

In Exps. 1-3, volatile compounds formed by the decomposition of the peroxides in oxidized samples were carried off by the nitrogen current, and passed succes-

sively through two traps containing a 5% solution of sodium carbonate to catch volatile acids and two traps containing a 0.2% solution of 2,4-dinitrophenylhydrazine in 2 *N*-hydrochloric acid to catch volatile carbonyl compounds. In Exps. 4-6, the nitrogen current carrying volatile compounds was passed first through two traps containing the solution of 2,4-dinitrophenylhydrazine to catch carbonyl compounds and then two traps containing a 5% solution of potassium carbonate, since there was some fear of causing changes of unstable carbonyl compounds by passing through a solution of alkali carbonate.

The solution in the traps for acids were concentrated by evaporation, acidified with a slight excess of hydrochloric acid, and extracted with relatively large amount of ether. The ether solution was washed with small amounts of saturated sodium chloride solution and then dehydrated with anhydrous sodium sulfate. On distilling off ether from the solution, there remained a light yellow liquid (volatile acids) having a pungent odor.

The precipitates of DNPH (2,4-dinitrophenylhydrazone) formed in the traps for carbonyl compounds were separated by filtration and dried under vacuum. Yields of volatile acids and DNPHs thus obtained are shown in Table 2.

TABLE 2. Yields of Volatile Compounds

Exp. No.	Sample (g.)	Volatile acid (g.)	DNPH (g.)
1	100 (I)	{(a) 0.2	(a) 0.07
		{(b) 0.3	(b) 0.10
		{(c) 0.8	(c) 0.26
		{(d) 0.7	(d) 0.12
2	107 (IV)	0.7	{(a) 0.25 {(b) 0.25
3	100 (IV)	0.4	{(a) 0.92 {(b) 1.14
4	200 (II)	0.8	0.45
5	400 (II)	0.9	{(a) 0.38 {(b) 0.21
6	400 (III)	1.3	{(a) 0.67
			{(b) 0.53

Notes: The yields (a), (b), (c) and (d) in Exp. 1 were determined separately for the volatile acids and DNPHs obtained, respectively, in 1st-7th, 8th-14th, 15th-24th and 25th-32nd oxidation-decomposition procedures. The yields (a) and (b) in Exps. 2 and 3 were determined separately for the DNPHs obtained, respectively, in the first half and latter half of oxidation-decomposition procedures. The yields (a) and (b) in Exps. 5 and 6 were determined separately for the DNPHs obtained, respectively, in the first and second traps.

## 2. Examination of Volatile Compounds

### (i) Volatile acids

Volatile acids in Table 2 were recognized as saturated acids since they did not react with iodine monochloride in glacial acetic acid. They were converted to hydroxamic acids, and the latter were analyzed by paper chromatography in ethanol, using butanol saturated with water as developer and a 10% solution of ferric chloride in ethanol as chromogenic reagent. The results are shown in Table 3.

TABLE 3. Components of Volatile Acids

Exp. No.	Sample	Acid						
		Formic	Acetic	Propionic	Butyric	Valeric	Caproic	
1	I	(a)	-	+	+	+	-	-
		(b)	-	+	+	+	-	-
		(c)	+	+	+	-	-	-
		(d)	+	+	+	-	-	-
2	IV	+	-	-	+	+	+	
3	IV	+	-	-	+	+	+	
4	II	+	+	-	-	+	+	
5	II	+	+	-	+	-	+	
6	III	+	+	+	+	-	+	

Notes: In each sample, component acids marked with positive sign were identified while those marked with negative sign could not be detected.

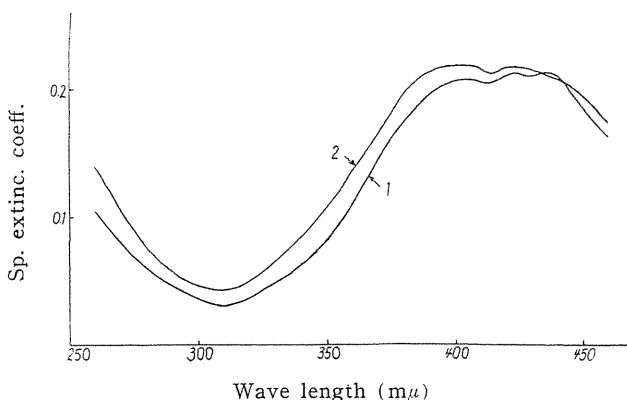
### (ii) Carbonyl compounds

The DNPHs in Table 2 were fractionated by column chromatography on alumina in the following way.

Exp. 1. The DNPH mixture was dissolved in benzene, and the solution was poured into an adsorption column packed with alumina and then developed with hexane-ether (97 : 3). The lower part of the column was colored orange-red while the middle part orange-yellow. There were several narrow bands of somewhat different colors on the upper part. The column of alumina was extruded and cut into 5, 4, 8 and 6 sections, respectively, for the samples (a), (b), (c) and (d) in Table 2. Each section was extracted with ether or ethyl acetate. Among the extraction fractions, those obtained in a sufficient amount were recrystallized from ethanol and then analyzed for their melting points, N-contents and ultraviolet absorption spectra. N-contents were found to be 23.2-20.1% (calcd. for propionaldehyde DNPH, 23.5%; calcd. for capraldehyde DNPH, 20.0%). Ultraviolet absorption spectra showed mostly absorption maxima at 360-370 m $\mu$  (saturated aldehyde DNPHs and  $\alpha$ ,  $\beta$ -unsaturated aldehyde DNPHs are reported to have absorption maxima at 356-358 m $\mu$  and 375-378 m $\mu$ , respectively<sup>3), 4), 5)</sup>). Most of the extraction fractions were considered to be mixtures of two or more DNPHs. The uniform DNPH fractions obtained were as follows: 2-hexenal DNPH of m.p. 142°-145°C, N 20.1% (calcd., 20.1%) and absorption maximum at 375 m $\mu$  from the sample (a); butyraldehyde DNPH of m.p. 121°-123°C and N 22.8% (calcd., 22.2%) from the sample (b); and butyraldehyde DNPH of m.p. 120°-122°C and N 21.8% from the sample (d).

When the combined filtrate freed from the precipitates of DNPH in Exp. 1 was stored for two months, a small amount of dark orange-red precipitates was formed. On fractionally crystallizing these precipitates from ethanol, the 1st crop melting at about 280°C and the 2nd crop of m.p. about 235°C and N 23.4% were obtained. Both crops developed a deep blue color with potassium hydroxide in ethanol, indicating the presence of dialdehyde DNPH<sup>5)</sup>. Ultraviolet absorption spectra of both crops are similar to that of dialdehyde DNPH<sup>3)</sup> as shown in Fig. 1.

Exps. 2-3. The DNPHs were dissolved in benzene. The solution was poured into the adsorption column packed with alumina, and then developed and eluted with the same solvent. After the lower zone of an orange-red color had been eluted, the column was extruded and cut into several sections, and each section



1. The 1st crop    2. The 2nd crop

FIG. 1. Absorption spectra of dialdehyde DNPHs.

was extracted with ethyl acetate. The fractions thus obtained were recrystallized or fractionally crystallized from methanol or ethanol to give eventually 5, 5, 8 and 11 fractions, respectively, from the samples (*a*) and (*b*) in Exp. 2 and the samples (*a*) and (*b*) in Exp. 3. Of these fractions, those obtained in a sufficient amount were analyzed for their melting points, N-contents and ultraviolet absorption spectra. The DNPH fractions obtained in Exp. 2 were found to consist mainly of DNPHs of saturated aldehydes of  $C_2-C_6$ . A fraction from the sample (*b*) in Exp. 2 was recognized as a crude butyraldehyde DNPH by its m.p.  $125^\circ-126^\circ C$ , N 22.2% (calcd., 22.2%) and absorption maximum at  $360 m\mu$ . The DNPH fractions obtained in Exp. 3 were found to contain DNPHs of saturated aldehydes of  $C_1-C_4$  together with DNPHs of unsaturated aldehydes consisting mainly of crotonaldehyde. A crude butyraldehyde DNPH fraction of m.p.  $119^\circ-120^\circ C$ , N 22.4% and absorption maximum at  $360 m\mu$  and a crude crotonaldehyde DNPH of m.p.  $180^\circ-182^\circ C$ , N 22.3% and absorption maximum at  $376 m\mu$  were obtained from the sample (*b*) in Exp. 3.

Exps. 4-6. The DNPHs were fractionated in a similar way as described for Exps. 2-3 to give eventually 11, 8, 8, 8 and 5 fractions, respectively, from the sample in Exp. 4, the samples (*a*) and (*b*) in Exp. 5 and the samples (*a*) and (*b*) in Exp. 6. Results of the analyses on fractions obtained in a sufficient quantity indicated that most fractions were mixtures of two or more aldehyde DNPHs. The saturated aldehyde DNPHs appeared to consist of DNPHs of  $C_2-C_5$  or  $C_1-C_6$  aldehydes. The unsaturated aldehyde DNPHs were found in all experiments, but the sample (*b*) in Exp. 5 and the sample (*b*) in Exp. 6 appeared to contain unsaturated aldehyde DNPHs in a relatively small proportion. Crotonaldehyde DNPH constituted the main component of unsaturated aldehyde DNPHs. A fraction of crotonaldehyde DNPH obtained in Exp. 4 had m.p.  $190^\circ-191^\circ C$ , N 22.4% (calcd., 22.4%) and absorption maximum at  $380 m\mu$ .

### 3. Discussion of Results

Although the yield of volatile compounds consisting of acids and carbonyls is expected to vary with the condition of the oxidation-decomposition of the sample,

the proportions of volatile acids and carbonyls (as DNPHs) in the volatile compounds are markedly different for each experiment. The yields of volatile acids in Exps. 4-6 may be a little lower than the real yields for the reason that some acids may remain in the traps for carbonyls and do not enter into the traps for acids since the volatile compounds were passed first through the traps for carbonyls. Apart from the yields in Exps. 4-6, the yields of volatile acids as compared with those of volatile carbonyls are remarkably high in Exp. 1 and relatively high in Exp. 2, while in Exp. 3 the total yield of volatile carbonyls is remarkably higher than the yield of volatile acids.

Also the compositions of volatile acids and carbonyls are somewhat different for each experiment. As shown in Table 3, none of the six saturated acids, formic to caproic, were identified throughout all experiments. Although it may be difficult to detect a saturated acid occurring in a very minor proportion in the sample by paper chromatography, the results shown in Table 3 indicate that the compositions of volatile acids differ for each experiment. Also the compositions of DNPHs differ for each experiment. While the previous study<sup>2)</sup> indicated the presence of DNPHs of three unsaturated aldehydes—crotonaldehyde, 2-pentenal and 2-hexenal—in the DNPH mixture obtained from the oxidation-decomposition products of highly unsaturated methyl esters, only 2-hexenal DNPH in Exp. 1 and crotonaldehyde DNPH in Exps. 3-6 could be separated in the present study. Such variance in the composition of volatile compounds is possibly due to the variance in the condition of oxidation-decomposition, but the detailed relationship between the composition of volatile compounds and the condition of oxidation-decomposition could not be known in the present study.

It is thus seen from the foregoing that although the volatile compounds obtained in the present study are not alike for each experiment, they contain, on the whole, saturated acids including formic to caproic acids, saturated aldehydes of C<sub>1</sub>-C<sub>6</sub> and unsaturated aldehydes such as crotonaldehyde and 2-hexenal. Some evidence for the presence of dialdehyde in the volatile compounds was indicated in the case of Exp. 1. As stated in the previous paper<sup>2)</sup>, every one of these volatile acids and carbonyls can not be regarded as the compound primarily responsible to the characteristic odor of the highly unsaturated acids of fish oil. The characteristic odor of the highly unsaturated acids of fish oil is considered to be attributable to some volatile compounds which could not be separated in the present study possibly for the reason that they are unstable and formed only in a minor amount as compared with the acids and carbonyls mentioned above.

#### 4. Summary

The highly unsaturated acids and their methyl esters prepared from sardine oil were oxidized by air or oxygen at room temperature, the oxidized samples were heated to effect the decomposition of the peroxides in the oxidized samples. The volatile compounds formed were examined with the results that although they were not alike depending on the condition of oxidation-decomposition, they contained, on the whole, saturated acids consisting of formic to caproic acids, saturated aldehydes of C<sub>1</sub>-C<sub>6</sub>, and unsaturated aldehydes such as crotonaldehyde and 2-hexenal. Some evidence for the presence of dialdehyde in the volatile compounds obtained in an experiment was indicated. As stated in a previous paper,

these volatile acids and carbonyls can not be regarded as the compounds primarily responsible to the characteristic odor of the highly unsaturated acids of fish oil. The characteristic odor of the highly unsaturated acids of fish oil is considered to be attributable to some volatile compounds which could not be separated and identified in this study possibly for the reason that these compounds are unstable and formed only in a minor amount as compared with the acids and carbonyls mentioned above.

#### References

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