

VOLATILE SUBSTANCES FORMED BY THE AIR OXIDATION OF THE METHYL ESTER OF HIGHLY UNSATURATED ACIDS

YOSHIYUKI TOYAMA and IWAO MATSUMOTO

Department of Applied Chemistry

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Fatty oils even once deodorized become sooner or later to have a disagreeable odor. This phenomenon is generally attributed to volatile substances formed by autoxidation of fatty oils. Recently, such volatile odoriferous substances from vegetable oils, such as cottonseed oil,¹⁾ corn and avocado oils,²⁾ soybean oil³⁾ and hydrogenated soybean oil,⁴⁾ have been separated by several authors. Since linolenic acid is considered to be a precursor of these volatile substances, Kawahara and Dutton⁵⁾ examined the volatile substances from autoxidized methyl linolenate. Regarding the disagreeable odor of refined fish oils, it was pointed out formerly by Tsujimoto⁶⁾ that the odor is to be attributed not to nitrogen compounds but to volatile odoriferous substances formed by autoxidation of highly unsaturated glycerides in fish oils. However, such volatile substances from fish oils have been little studied until recently. Nozu and his coworkers⁷⁾ studied on the odoriferous substances of whale oil with the results that neither nitrogen compounds nor sulfur compounds participate in odor development and that the odor is attributed to volatile free fatty acids, carbonyl compounds and peroxides, among which formic, propionic, butyric and isovaleric acids, 2-heptenal, formaldehyde, propanal together with some compounds, possibly aldehyde-acids and aldehyde-peroxides, are found. In the first step of our scheme to gain a further information on the odoriferous substances from fish oils, the present paper deals with the volatile substances formed by the air oxidation of the methyl ester of highly unsaturated acids which appear to be the essential precursor of fish oil odor.

1. Sample

A highly unsaturated acid concentrate separated from sardine oil by means of the sodium salt acetone method was converted into methyl ester. Fractionation of the methyl ester yielded a fraction of B.P. 180°-216° C/ca. 1 mm, n_D^{15} 1.4856, A.V. 0.28, S.V. 176.3 and I.V. (pyridine sulfate dibromide method) 340.3. These characteristics indicate that this fraction consists chiefly of the methyl ester of highly unsaturated C₂₀ and C₂₂ acids.

2. Oxidation of Highly Unsaturated Methyl Ester and Collection of Volatile Substances

A glass tube of 3.5 cm diameter and 46 cm length was packed with fine pieces of pumice, and impregnated with 100 g of the highly unsaturated methyl ester

obtained above. The tube was horizontally placed on a water bath so that the inside of tube was kept at 45°-55° C. A current of air was passed into the tube at a rate of about 10 liters per hour. The volatile substances formed by oxidation of the methyl ester were carried off by the air current and passed through a series of traps (Fig. 1). The trap *B* contains a 5% solution of sodium carbonate. The traps *C* and *D* contain a 10% solution of sodium bisulfite. The trap *E* contains a 0.2% solution of 2, 4-dinitrophenylhydrazine acidified with hydrochloric acid. The final trap *F* is leer and cooled with ice. But there remained scarcely any substance in the trap *F* after the experiments. The cooler *K* mounted on each trap is cooled with cold water. The air current coming out of the trap *F* had scarcely any pungent odor. After the oxidation for 150 hours, the content of tube *A* and the solutions of traps *B*, *C* and *D* were renewed. Repeating these procedures four times, 400 g of the methyl ester in total was treated.

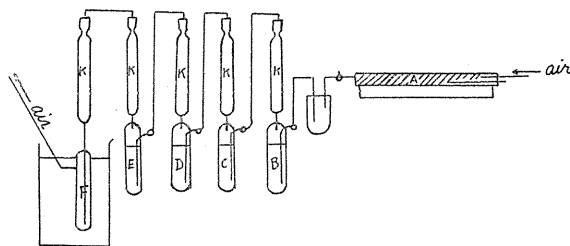


FIG. 1

3. Examination of Volatile Substances

a. Volatile acids. The solution (about 250 cc) obtained in the trap *B* was concentrated to about 50 cc, acidified with a slight excess of hydrochloric acid, and extracted with relatively large amount of ether. After distillation of ether from the ether solution there remained about 2 g of a light yellow liquid having a pungent odor resembling acetic acid. This was converted into hydroxamic acids,⁵⁾ and the latter were analyzed by paper chromatography in ethanol with the results shown in Table 1. An inspection of R_f values indicated the presence of formic, acetic, butyric and heptanoic (or caproic) acids. Based on the color intensity of the respective spots, acetic acid appeared to be present in the largest proportion, followed by butyric, formic and heptanoic acids in descending order.

TABLE 1

Hydroxamic acid from	R_f (Descending method)		Coloration
	Pure sample	Sample under examination	
Formic acid	0.42	0.41	Purple
Acetic acid	0.53	0.53	"
Butyric acid	0.81	0.80	"
Caproic acid	0.93		
Heptanoic acid	0.95	0.94	"

Filter paper: "Toyo Filter Paper No. 50"; developer: butanol saturated with water; temperature: 15°-20° C; chromogenic reagent: 10% solution of ferric chloride in ethanol.

b. Carbonyl compounds. The solution of sodium bisulfite (about 500 cc) obtained in the traps *C* and *D* was concentrated to about 100 cc volume, and a 10% solution of potassium hydroxide was added in a slight excess. The carbonyl compounds liberated were extracted with ether. The ether solution was washed with a saturated solution of sodium chloride, dehydrated and distilled, yielding 0.5 g of a light yellow liquid having a heptanal-like pungent odor. It absorbed little bromine in chloroform. Twenty cc of water was added to it, and the mixture was oxidized with a slight excess of potassium permanganate under cooling with ice. After the completion of oxidation, the acidic oxidation product was separated in the usual way by using ether. Hydroxamic acids prepared from the acidic oxidation product were analyzed by paper chromatography with the results shown in Table 2. The R_f values indicated the presence of acetic, butyric and heptanoic acids. Based on the intensity of coloration, heptanoic acid appeared to be largest in quantity. Hence the carbonyl compounds collected in the traps *C* and *D* contain heptanal together with acetaldehyde and butyric aldehyde. It is, however, to be noted that most of acetaldehyde should have been carried off with ether during the distillation of the ether solution of carbonyl compounds.

TABLE 2

Hydroxamic acid from	R_f (Descending method)		Coloration
	Pure sample	Sample under examination	
Acetic acid	0.53	0.53	Purple
Butyric acid	0.81	0.82	"
Caproic acid	0.93		
Heptanoic acid	0.95	0.95	"

Notes: The experiments of Table 2 were carried out in the same way as the experiments in Table 1.

The solution (250 cc) obtained in the trap *E* deposited precipitates. The mixture was extracted successively with hexane, ether and ethanol. The hexane extract yielded a yellow crystalline solid which, after recrystallization, showed M.P. 121° C and Mol. Wt. (Rast) 252. 2,4-Dinitrophenylhydrazone of butyric aldehyde has M.P. 123° C⁹⁾ and Mol. Wt., Calc. 252. The ether extract showed M.P. 75° C after recrystallization from ethanol, but scarcity of material did not permit further studies. The ethanol extract appeared to be somewhat unstable, forming an ethanol insoluble product of dark red color on standing in the air.

4. Discussion

As described above, formic, acetic, butyric and heptanoic (or caproic) acids as volatile acids and acetaldehyde, butyric aldehyde and heptanal as carbonyl compounds have been identified among the volatile substances formed by oxidation of the methyl ester of highly unsaturated acids. All these compounds have a pungent odor, but they can not be considered to be the essential components of odoriferous substances formed on standing highly unsaturated acids or their esters in the air. The ether extract from the substances obtained in the trap *E* appear to contain 2,4-dinitrophenylhydrazone of some unsaturated carbonyl compounds. If the essential components of odoriferous substances are such unstable carbonyl compounds,

it would be necessary to carry out the oxidation of highly unsaturated methyl ester under a very mild condition without prolonged exposure to air in order to identify such unstable carbonyl compounds without being suffered further oxidation.

Summary

The methyl ester of highly unsaturated acids prepared from sardine oil has been air-oxidized at 45°-55° C. Among the volatile oxidation products, formic, acetic, butyric and heptanoic (or caproic) acids, acetaldehyde, butyric aldehyde and heptanal have been identified. Also the presence of some carbonyl compounds, possibly unsaturated carbonyl compounds, yielding unstable 2,4-dinitrophenylhydrazone has been indicated.

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