Autoxidation Rates of 5-Olefinic Monoenoic and Dienoic Fatty Acids from Sea Urchin Lipids and Meadowfoam Oils

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Autoxidation rates of the 5-olefinic monoenoic and dienoic fatty acids from sea urchin lipids and meadowfoam oils were compared with those of normal monoenoic and dienoic fatty acids by gas-liquid chromatographic determination of the unoxidized fatty acid methyl esters remaining through the autoxidation period. The fatty acids are classified into five groups shown below according to the oxidation rate of their methyl esters: I 5-olefinic monoenoic acids (c5-18:1, c5-20:1 and c5-22:1), II normal monoenoic acids (c9-18:1, c11-18:1, c9-20:1, c13-20:1 and c13-22:1), III 5-olefinic dienoic acids (c5,c11-20:2, c5,c13-20:2 and c5,c13-22:2), IV 7-olefinic dienoic acids (c7,c13-22:2 and c7,c15-22:2) and V normal dienoic acids (c6,c9-18:2, c9,c12-18:2 and c11,c14-20:2). The oxidation rates of these groups increased during autoxidation in order from I to V. These results show that the 5-olefinic monoenoic and dienoic acids are more stable to autoxidation than the normal monoenoic and dienoic acids, respectively. The higher stabilities of the 5-olefinic monoenoic and dienoic acids in organisms are shown from these results.

Wide distribution of the unusual 5-olefinic acids has been found in the lipids of echinodermata (1-6), molluscs (7-10), marine algae (11-13), meadowfoam and other plants (14-18). In our previous paper (19), it has been shown that autoxidation rates of the typical 5-olefinic nonmethylene-interrupted (NMI) polyenoic acids in natural sources were much lower than those of methylene-interrupted (MI) polyenoic acids having the same double bond number.

In this study, autoxidation rates of the methyl esters of the 5-olefinic monoenoic and dienoic acids from sea urchin lipids and meadowfoam oils, c5-18:1, c5-20:1, c5-22:1, c5,c11-20:2, c5,c13-20:2 and c5,c13-22:2 were compared with those of the normal monoenoic and dienoic acids. Information on the autoxidation of the 5-olefinic monoenoic and dienoic acids reported in this study will be helpful in further investigations of these 5-olefinic acids.

MATERIALS AND METHODS

Materials. The sample of sea urchin *Strongylocentrotus nudus* was obtained from subtidal water near Hakodate. The total lipids were extracted from gonads of *S. nudus* by the method of Bligh and Dyer (20) and converted to fatty acid methyl esters (FAME) by refluxing with 5% CH_3COCl-MeOH for three hr. FAME were recovered by hexane extraction and purified by silicic acid column chromatography (Kieselgel 60, Merck, Darmstadt, West Germany) with ether/hexane (1:9, v/v) for elution. Methyl esters of the 5-olefinic monoenoic and dienoic acids were concentrated by argentation column chromatography using 20% $AgNO_3$-silica gel as the packing, and ether/hexane as the solvent for elution. The percentages of ether in the solvents were progressively increased from 0 to 40%.

The meadowfoam oils (Nikko Chemical Co., Tokyo, Japan) and olive oils (Nakarai Chemical Co., Kyoto, Japan) were converted to FAME by the method described above.

Oxidation of the methyl esters. Each 300 mg of the methyl esters of concentrates of the 5-olefinic acids from sea urchin lipids and mixtures of meadowfoam oils and olive oils (2:1, w/w) in flat-bottomed glass tubes (capacity 10 ml and bottom diameter 19 mm i.d.) were autoxidized by incubation in the dark at 50°C. One μl of sample for determination of the unoxidized substrate contents was taken from the oxidized samples at certain time intervals throughout the oxidation period. The unoxidized methyl esters were separated from the oxidized substrates by thin layer chromatography (TLC) with Silica Gel G plates of 0.5 mm thickness by developing with hexane/ether (85:15, v/v), and subjected to gas-liquid chromatography (GLC) by the method described below.

GLC. Open tubular GLC of the methyl esters was done with a Shimadzu GC6AM instrument (Shimadzu Seisakusho Co., Kyoto, Japan), with a dual flame ionization detector (FID) on a wall-coated open-tubular glass column coated with SP 2300 (50 m × 0.3 mm i.d.). The column temperature was 190°C and the injector and detector were held at 230°C. Peak area percentages were obtained with a Shimadzu integrator C-R2AX. The unoxidized substrate contents were calculated from the peak area ratios of the substrate methyl ester to stearic acid (18:0) methyl ester.

RESULTS AND DISCUSSION

Oxidation rates of the 5-olefinic acids. The fatty acid compositions of the samples oxidized in this study, and their sources, are shown in Table 1, and the changes in the unoxidized substrate contents and the oxidation rates of the methyl esters of fatty acids from sea urchin lipids are shown in Table 2, Figures 1 and 2.

The seed oils of meadowfoam contain large amounts of c5-20:1, c5-22:1 and c5,c13-22:2 and small amounts of c5-18:1 (Table 1). The oxidation rates of the methyl esters of the 5-olefinic acids and normal acids of meadowfoam oils were investigated in the same way as the sea urchin lipids. Because meadowfoam oils contain only slight amounts of normal monoenoic acids for comparison of their oxidation rates with the 5-olefinic acids, the methyl esters of olive oils containing about 80% oleic acid were added to the methyl esters of meadowfoam oils used for the investigation. The results are shown in Table 3 and Figure 3.

On the basis of these results, the fatty acids can be classified into five groups shown below according to the oxidation rates of their methyl esters: I 5-olefinic monoenoic acids (c5-18:1, c5-20:1 and c5-22:1); II normal monoenoic acids (c9-18:1, c11-18:1,

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TABLE 1

Compositions of the Fatty Acid Methyl Esters of the Samples for Oxidation and Their Sources (wt%)

		Samples			
		A Sea urchin total lipids	B Concentrate of the 5-olefinic acids of sea urchin	C Meadowfoam oils	D Mixture of meadowfoam oils and olive oils (2:1, w/w)
Peak no.	Fatty acid	(Source)	(For oxidation)	(Source)	(For oxidation)
1	14:0	10.86	10.82	—	—
2	16:0	13.73	14.21	0.23	2.88
3	9-16:1	2.36	5.56	—	—
4	*iso*-17:0	2.57	5.14	—	—
5	18:0	1.93	1.62	0.14	0.98
6	5-18:1	0.79	1.44	0.46	0.28
7	9-18:1	1.62	3.10	1.38	25.14
8	11-18:1	2.45	5.15	—	—
9	18:2(n-9)	1.04	2.13	—	—
10	18:2(n-6)	0.88	2.33	1.15	2.05
11	18:3(n-3)	1.89	0.96	1.19	0.61
12	18:4(n-3)	6.10	—	—	—
13	20:0	0.65	0.45	0.39	0.58
14	5-20:1	4.05	6.46	59.34	42.00
15	9-20:1	0.96	2.24	—	—
16	11-20:1	2.99	5.10	2.45	1.06
17	13-20:1	0.79	1.55	—	—
18	5,11-20:2	5.21	12.78	—	—
19	5,13-20:2	1.29	3.49	—	—
20	20:2(n-9)	0.93	1.89	—	—
21	20:2(n-6)	2.13	3.81	—	—
22	5,11,14-20:3	0.15	0.19	—	—
23	20:4(n-6)	9.34	—	—	—
24	5,11,14,17-20:4	0.16	—	—	—
25	20:5(n-3)	13.43	—	—	—
26	5-22:1	—	—	3.60	2.29
27	11-22:1	0.08	0.24	—	—
28	13-22:1	1.35	2.79	9.65	8.05
29	5,13-22:2	—	—	20.03	14.08
30	7,13-22:2	0.30	0.84	—	—
31	7,15-22:2	0.86	2.65	—	—
32	22:6(n-3)	0.42	—	—	—
	Others	8.67	3.04	—	—

c9-20:1, c13-20:1 and c13-22:1); III 5-olefinic dienoic acids (c5,c11-20:2, c5,c13-20:2 and c5,c13-22:2); IV 7-olefinic acids (c7,c13-22:2 and c7,c15-22:2), and V normal dienoic acids (c6,c9-18:2, c9,c12-18:2 and c11,c14-20:2). These results obtained from different sources of the 5-olefinic acids suggested that the 5-olefinic acids were more stable than other normal fatty acids having the same double bond number. In our previous paper (19), it was reported that the oxidation rates of NMI dienoic and trienoic acid methyl esters (c5,c9-18:2, c5,c9,c12-18:3 and t5,c9,c12-18:3) were lower than those of MI dienoic and trienoic acid methyl esters (c9,c12-18:2 and c9,c12,c15-18:3), respectively. In this study, the oxidation rates of NMI dienoic fatty acid methyl esters (c5,c11-20:2, c5,c13-20:2 and c5,c13-22:2) also were much lower than those of MI dienoic fatty acid methyl esters (c6,c9-18:2, c9,c12-18:2 and c11,c14-20:2). It has been shown that the 5-olefinic NMI dienoic acids were slightly more stable for the autoxidation than the 7-olefinic NMI dienoic acids by comparison of the oxidation rates of the methyl esters of the 5-olefinic NMI dienoic acids and the 7-olefinic NMI dienoic acids from sea urchin lipids (Fig. 2).

We reported that the methyl esters of the 5-olefinic NMI and normal polyenoic acids oxidized more slowly as mixtures than as a single substrate and the autoxidation of the methyl esters of these acids was accelerated by the oxidation promotive effect of free radicals formed in the autoxidation of the other substrates which were more susceptible to autoxidation (19). The samples used in this study were mixtures; it has been presumed that the autoxidation of the 5-olefinic acids

TABLE 2

Comparison of Autoxidation Rates of Methyl Esters of Fatty Acids in Sea Urchin Lipids (Sample B[a]) at 50°C

Group	Fatty acid	Loss of unoxidized esters at 800 hr (%)	Loss of unoxidized esters at 1,000 hr (%)
I	c5-20:1	28.4	54.0
II	c9-18:1	32.6 Av[b] 35.0	61.2 Av 61.3
	c11-18:1	33.7	60.3
	c9-20:1	36.4	61.0
	c13-20:1	37.3	62.7
III	c5,c11-20:2	49.9 Av 50.2	79.7 Av 79.7
	c5,c13-20:2	50.4	79.7
IV	c7,c13-22:2	52.7 Av 53.7	83.9 Av 83.5
	c7,c15-22:2	54.7	83.1
V	c6,c9-18:2	97.7 Av 98.1	100.0 Av 100.0
	c9,c12-18:2	98.1	100.0
	c11,c14-20:2	98.4	100.0

[a]See Table 1.

[b]Av, the average value of each group.

TABLE 3

Comparison of Autoxidation Rates of Methyl Esters of Fatty Acids in the Mixture of Meadowfoam Oils and Olive Oils (Sample D[a]) at 50°C

Group	Fatty acid	Loss of unoxidized esters at 1,300 hr (%)	Loss of unoxidized esters at 1,600 hr (%)
I	c5-18:1	31.7 Av[b] 30.7	59.5 Av 58.1
	c5-20:1	30.2	56.6
	c5-22:1	30.1	58.3
II	c9-18:1	34.8 Av 35.5	63.8 Av 63.9
	c13-22:1	36.1	64.0
III	c5,c13-22:2	54.2	84.3
V	c9,c12-18:2	98.5	100.0

[a]See Table 1.

[b]Av, the average value of each group.

will be retarded in single substrate rather than mixture.

Specificities of the 5-olefinic acids. Autoxidative stability of the 5-olefinic acids elucidated in this study probably is due to the influence of the carboxyl group. Influences of the carboxyl and ester group often were observed in ^{1}H- and ^{13}C-nuclear magnetic resonance analyses of fatty acids and their methyl esters as a deshielding effect or chemical shift parameter (21,22). It was shown in these papers that these functional groups affected electron density of carbon or hydrogen atoms until the Δ8 position. It is contemplated that these influences of carboxyl and ester groups retard formation of the allylic radical of 5-olefinic acid methyl esters. Frankel et al. reported (23) that hydroperoxide is liable to be formed at the allylic carbon of the Δ15 double bond which is closer to the methyl terminal in the autoxidation of c9,c15-18:2.

Some previous papers reported that fatty acids having double bonds near the carboxyl group, especially 5-olefinic bond, had unusual properties for several reactions. Heimermann et al. (24) showed that c5-18:1 esters were most resistant in pancreatic lipolysis of the 18:1 isomers, and Bottino et al. (25) showed that 20:5(n-3) having a 5-olefinic bond was also resistant to pancreatic lipolysis. Holman et al. (26) showed

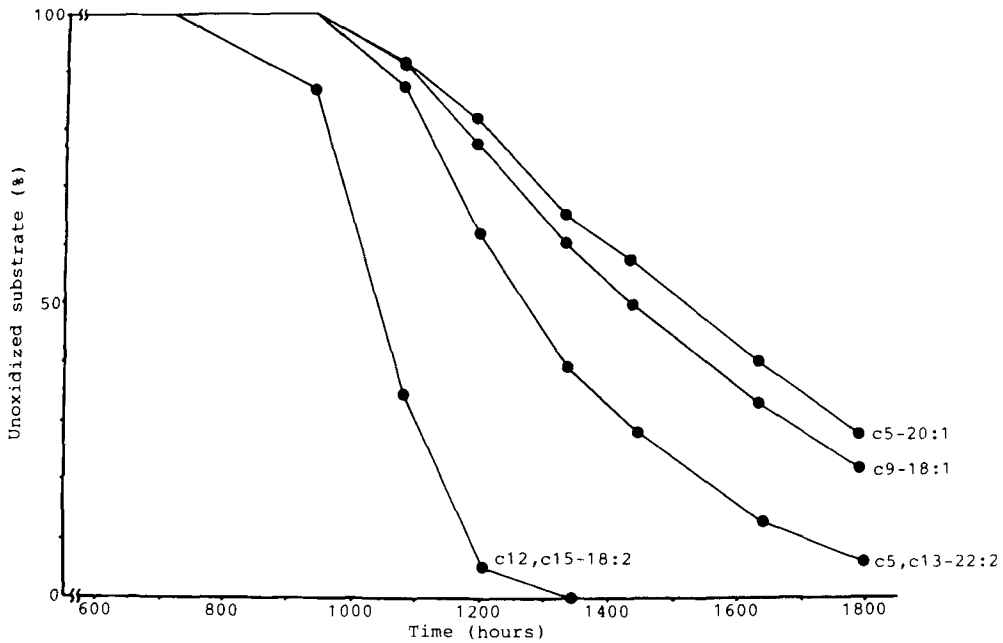

FIG. 3. Changes in the amount of unoxidized substrate during autoxidation of the methyl esters of the mixture of the meadowfoam oils and olive oils (Sample D; see Table 1).

that the fatty acids containing the Δ2 through Δ6 isomers of MI *cis,cis*-octadecadienoic acids were hardly oxidized by the catalysis of soybean lipoxygenase.

The 5-olefinic acids are included mainly as the acyl components in the polar lipids and the biomembranes of sea urchins (1,3), and it is suggested that the 5-olefinic acids contribute fluidity to the membranes. The stabilities of 5-olefinic acids for several reactions are available for maintenance of the membranes, and it is suggested that the 5-olefinic acids constitute stable membranes.

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