Fatty Acids in Echinoidea: Unusual *cis*-5-Olefinic Acids as Distinctive Lipid Components in Sea Urchins

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Open tubular gas liquid chromatographic (GLC) analyses of fatty acids from total lipids of 12 species of Echinoidea collected at several locations along the Pacific coast of Japan showed the same unusual *cis*-5-olefinic acids in all species, i.e., *cis*-5-octadecenoic acid (5-18:1), *cis*-5-eicosenoic acid (5-20:1), all-*cis*-5,11- and 5,13-eicosadienoic acids (5,11- and 5,13-20:2), all *cis*-5,11,14-eicosatrienoic acid (5,11,14-20:3) and all-*cis*-5,11,14,17-eicosatetraenoic acid (5,11,14,17-20:4). The structural analysis of partially purified 5,11,14,17-20:4 was undertaken by reductive ozonolysis with GLC and gas chromatographic-mass spectrometric analyses of the products. ^{13}C-Nuclear magnetic resonance analyses of the totals and fractions of fatty acid methyl esters from the sea urchin lipids did not show any occurrence of fatty acids having an isolated olefinic bond in the 2, 3 or 4 positions. The 5-olefinic acids were concentrated on the polar lipids rather than neutral lipids. The branched and odd chain fatty acid contents of mud-feeding sea urchins were found to be relatively greater proportions of total fatty acids than in algae feeders.
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The fatty acid details of sea urchin *Strongylocentrotus droebachiensis* harvested near Halifax, Nova Scotia, Canada, have been reported previously (1). The occurrence of unusual 5-olefinic acids was noticeable in all samples, amounting to as much as 10–21% of fatty acids from the total lipids of whole animals. The major components found were 5-18:1, 5-20:1, 5,11- and 5,13-20:2. In a subsequent study, fatty acids from six species of sea urchins collected in Japan were investigated, and the occurrence of the 5-olefinic acids was similar to that in Atlantic sea urchins (2,3).

In this study, fatty acids of lipids from 12 species of Echinoidea obtained in Japan have been investigated in greater detail. The 5-olefinic acids were found in the lipids of all of the samples, and their occurrence is thus established as a common and characteristic feature of Echinoidea lipids. The 5,11,14,17-20:4 and 5,11,14-20:3 were found to be minor ($\leq$0.5%) components of the total fatty acids. The former has been found in seed lipids of some gymnospermae (4), but has not been reported as a fatty acid component of animals.

MATERIALS AND METHODS

Materials. The species of Echinoidea studied are listed in Table 1. Samples 1, 5, 6, 8, 9 and 11 were obtained in Kanagawa prefecture; 3, 4, 7, 10 and 12 at Hokkaido; and 2 in Okinawa prefecture. Samples were collected from shallow subtidal water, except samples 3, 4 and 7 (50 m off Hakodate), 10 (50 m off Sarufutsu), and 9 (740 m in Sagami Bay).

Salted gonads of sea urchins *S. intermedius* and *S. nudus* were obtained at a food market in Hakodate.

All samples were kept frozen at -20 C in MeOH for a few months until used.

Preparation of methyl esters. The total lipids of sea urchins and gonads were extracted by the method of Bligh and Dyer (5). Fractionation of total lipids (TL) into neutral (NL) and polar (PL) lipids was carried out by column chromatography using silicic acid (Kiesel Gel 60, Merck, Darmstadt, Federal Republic of Germany) with chloroform and methanol as the developing solvents. The lipids were converted to fatty acid methyl esters by direct transesterification with 5% HCl solution in methanol by heating at 80 C in screw-cap test tubes for 3 hr under nitrogen. The methyl esters were separated from other products by thin layer chromatography (TLC) with Silica Gel G plates of 0.5 mm thickness by developing with n-hexane/ether (85:15, v/v).

Gas liquid chromatography (GLC). Open tubular GLC of the methyl esters was done with Shimadzu GC 6AM and 7A instruments (Shimadzu Seisakusho Co., Kyoto, Japan), with an FID detector, on wall-coated open-tubular glass columns coated with SP 2300 (50 m $\times$ 0.3 mm id). The column temperatures were 175 or 180 C and the injector and detector were held at 230 C. Peak area percentages were obtained with Shimadzu integrators C-R1A and C-R2AX.

^{13}C-Nuclear magnetic resonance (^{13}C- NMR). A JEOL FX-90Q spectrometer (Nippon Denshi Co., Tokyo, Japan) in the Fourier transform mode at 22.5 MHz for ^{13}C was used to obtain the ^{13}C-NMR spectrum of methyl esters in $CDCl_3$.

Argentation chromatography. The trienoates and tetraenoates were concentrated by argentation column chromatography using 20% $AgNO_3$-silica gel as the packing and ether/n-hexane mixtures as the eluting solvents. The percentage of ether in the mixtures was progressively increased from 2 to 100%.

The separation of the trienoates and tetraenoates was carried out by preparative argentation-TLC ($AgNO_3$-TLC) on $AgNO_3$-impregnated layers of Silica Gel G by developing with ethyl acetate/n-hexane (1:9, v/v) for the trienoates and (3:7, v/v) for the tetraenoates.

Reductive ozonolysis. Reductive ozonolysis of 1–5 mg methyl ester samples was carried out in methylene chloride at -70 C using the procedure of Kleiman et al. (6). The ozonides were reduced by addition of a few crystals of triphenylphosphine. Aldehydic products were analyzed by GLC using a 2 m $\times$ 3 mm id glass column packed with 3% Silar 10C on Gas Chrom Q (100–120 mesh). The column temperature was programmed from 60 to 280 C at 2 C/min. The weight percentages were calculated from the peak area percentages using C-factor (7). The detector and injector temperatures were kept at 300 C. The carrier gas was nitrogen at 30 ml/min.

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Gas chromatography-mass spectrometry (GC-MS). GC-MS of the ozonolysis products was carried out with a Hitachi M-60 GC-MS system containing a 2 m × 3 mm id glass column packed with 3% Silar 10C on Gas Chrom Q (100–120 mesh), using helium gas as the carrier gas. All mass spectra were obtained at 20 eV ionization electron energy and a source temperature of 180 C.

RESULTS AND DISCUSSION

Unusual fatty acids. In our previous paper (1), three unusual 5-olefinic acids, 5-20:1, 5,11- and 5,13-20:2, were identified as major and unusual components of the Atlantic sea urchin *S. droebachiensis*. Their structures were determined by GLC of the ozonolysis products, mass spectroscopic analysis of the pyrrolidides and other procedures. A small component was tentatively identified as 5,11,14-20:3 by comparisons of GLC retention data.

A typical gas chromatogram of the fatty acid methyl esters (C_{20} fraction) obtained in this study is shown in Figure 1A. All peaks found in the previous paper (1) were also observed in the chromatograms obtained in this study. An additional peak "b" for an unknown minor component was noted between the peaks of 20:3(n-3) and 20:4(n-3).

Large-scale fractionation of polyenoic acids. To facilitate identification of peak "b," which was common to all samples, a large-scale fractionation of fatty acids from a readily available sea urchin material was undertaken. Total lipids (9.78 g) were extracted from 100 g of salted gonads of *S. intermedius* (sample 3) and *S. nudus* (sample 4). The fatty acids (7.27 g) obtained from these lipids were fractionated by urea adduct methods using urea (20 g) and methanol (100 ml). The polyenoic acid fraction (3.94 g) recovered from the filtrate was converted to methyl esters. The methyl esters of polyenoic acids separated by argentation column chromatography, and the trienoate and tetraenoate fractions were further fractionated by $AgNO_3$-TLC. The gas chromatograms of the trienoate and tetraenoate fractions obtained are shown in Figures 1B and 1C. Peaks "a" and "b" in each figure were identified as 5,11,14-20:3 and 5,11,14,17-20:4 on the basis of the agreement of the retention data with those of the reference methyl esters of those acids obtained from *Podocarpus nagi* seed and *Juniperus chinensis* seed (4), respectively. These results were confirmed by open tubular GLC analyses on another liquid phase SP-2340 (50 m × 0.3 mm id; column temperature 160 C; injector and detector temperature 230 C).

The tetraenoate fraction could be separated into two fractions by $AgNO_3$-TLC, using ethyl acetate/n-hexane (3:7, v/v) as the developing solvent. Fraction 1 (Rf = 0.44) contained 46.3% 20:4(n-6) and 53.7% 20:4(n-3). Fraction 2 (Rf = 0.34) contained 1.3% 20:4(n-6), 53.8% 20:4(n-3) and 44.9% "b" (5,11,14,17-20:4). The compositions of these two fractions indicate the order of mobility from the top to the bottom to be 20:4(n-6), 20:4(n-3) and "b" (5,11,14,17-20:4).

Products from reductive ozonolysis. Since complete separation of individual 20:3 and 20:4 acids was impossible, fractions 1 and 2 were subjected to ozonolysis followed by GLC of the products. Some component peaks were identified on the basis of the agreement of the retention times with those of authentic reference esters and through oxidative fission products. Thus, reductive ozonolysis products from 5,11,14-20:3 contained n-hexanal (I), 1,6-hexanedial (II) and methyl 5-oxopentanoate (III); those from 5,11,14,17-20:4 contained II and III; those from 20:4(n-3) contained methyl 8-oxooctanoate; and those from cyclohexene contained II. The compositions of the ozonolysis products from fractions 1 and 2 are shown in Table 2. The fair agreement of the proportions of mole percentages (II/III) between fraction 2 and reference acid 5,11,14,17-20:4 (Table 2) characterized peak "b" as 5,11,14,17-20:4.

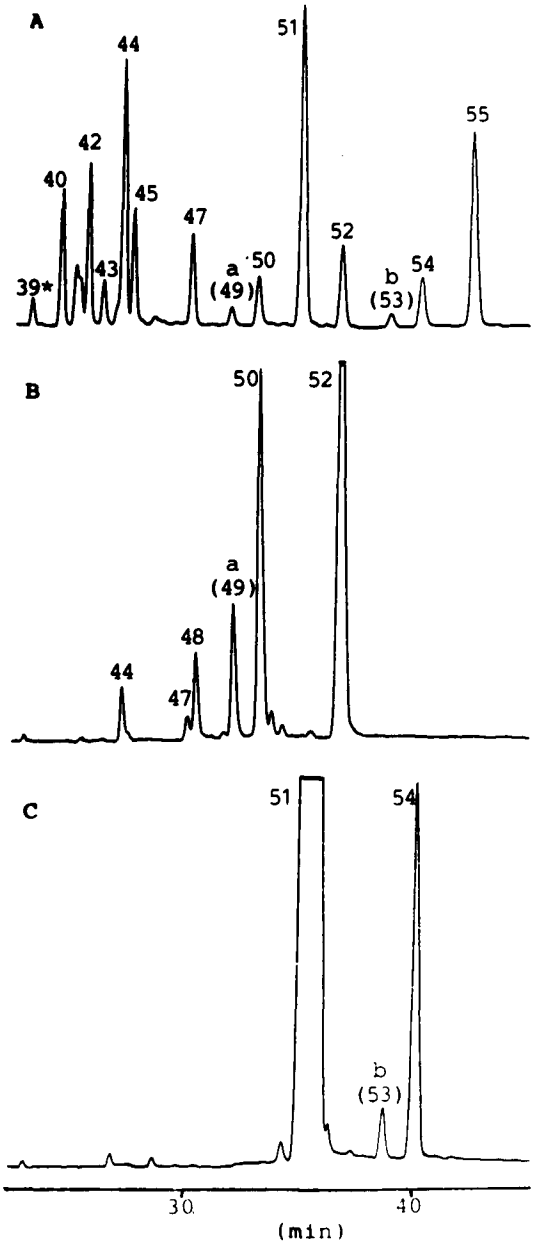

FIG. 1. Parts of gas chromatogram of fatty acid methyl esters of the total lipids from *H. pulcherrimus*. A, Total; B, trienoate fraction; C, tetraenoate fraction. *, See Table 4.

GC-MS of ozonolysis products. The mass spectra of the hexanedial fraction from the ozonolysis products of the 5,11,14,17-20:4 concentrates were taken by the GC-MS

TABLE 1

Echinoidea Samples and Their Contents of Lipids and 5-Olefinic Acids

No.	Order	Genus and species	Lipids (%)[a]	5-Olefinic acids (%)[b]	Date obtained
1	Echinoida	*Anthocidaris crassispina*	5.20	11.13	July 80
2	Echinoida	*Echinometra mathaei*	0.50	5.90	August 83
3	Echinoida	*Strongylocentrotus intermedius*	0.56	19.18	March 84
4	Echinoida	*Strongylocentrotus nudus*	0.58	21.14	March 84
5	Echinoida	*Hemicentrotus pulcherrimus*	1.84	11.39	July 80
6	Echinoida	*Pseudocentrotus depressus*	5.30	9.88	August 80
7	Arbacioida	*Glyptocidaris crenularis*	1.12	10.88	Feb. 84
8	Diadematoida	*Diadema setosum*	2.10	9.07	August 83
9	Cassiduloida	*Echinolampas sternopetala*	1.00	14.49	August 80
10	Spatangoida	*Echinocardium cordatum*	0.30	12.44	August 83
11	Clypeasteroida	*Clypeaster japonicus*	0.64	8.38	July 81
12	Clypeasteroids	*Scaphechinus mirabilis*	0.60	12.54	August 79

[a]Wt %: total lipids to a wet sample.
[b]Wt %: all 5-olefinic acids in total fatty acids.

instrument. The mass to charge ratio of the fragment ions and their intensities are shown in comparison with those of an authentic reference sample in Table 3. The agreement further supported the occurrence of 5,11,14,17-20:4 in the sea urchin lipids. In the assignment of the ions in Table 3, the mass numbers of the fragment ions were attributed to the loss of the following fragments: 18 ($H_6O^{\bullet}$), 29 ($HCO^{\bullet}$), 44 ($CH_2=CHOH^{\bullet}$), 57 ($OHCCH_2CH_2^{\bullet}$) and 70 ($CH_2=CH\text{-}CH_2\text{-}CHO^{\bullet}$). The (M-70) and (M-57) ions were formed by the fragmentation process, including McLafferty rearrangement, and the scission of the central C_3-C_4 bond, respectively.

^{13}C-NMR, infrared (IR) and ultraviolet (UV) analyses. Previously, Kochi reported occurrence of 3,11-20:2 as a component of roe lipids of sea urchins obtained in Japan and Korea (8,9). To investigate the occurrence of 3-olefinic acids and any other acids having an olefinic bond near the ester group, the ^{13}C-NMR spectra of the original acid methyl esters and their fractions were examined. No signals were found at the chemical shifts of *cis*- and *trans*-2- to 4-olefinic acids expected from literature data (10) as shown in Figure 2. The characteristic signals of the *cis*-5-olefinic acids shown in Figure 2 were in accord with those reported in previous papers (4,10). This result also supported the occurrence of the 5-olefinic acids in the sea urchins. IR spectra showed no absorbance near 971 cm^{-1}, and UV spectra showed no absorbance near 232 nm, 270 nm and 302 nm, respectively. These results showed the absence of *trans* and conjugated unsaturated compounds in methyl esters of the sea urchins (11).

Unusual 5-olefinic acids. The compositions of the fatty acids from the total lipids of Echinoidea are shown in Table 4, and the gas chromatogram of fatty acid methyl esters of total lipids from *Pseudocentrotus depressus* is shown in Figure 3. All the samples contained about 6–20% of the 5-olefinic acids. The results are similar to those of the Atlantic sea urchin *S. droebachiensis* (1), which showed somewhat higher percentages of total 5-olefinic acids (10–20%).

It is noteworthy that all samples of Echinoidea in Table 4 show very significant contents of the 5-monoenoic acids (2.3–9.3% to the total fatty acids). The 5-monoenoic acids have been reported as minor components of certain marine invertebrates, such as periwinkle *Littorina littorea*, moon snail *Lunatia triseriata* and sand shrimp *Crangon septemspinosus*, but at levels of 0.2% or less (12).

TABLE 2

Compositions of Ozonolysis Products from the 20:4 Fraction and a Sample of Authentic 5,11,14,17-20:4 from *Juniperus chinensis* Seed

	Products[a]			
	6A	5AE	6AA	8AE
RT[b] (min)	3.65	28.03	34.87	43.93
Fraction 1[c]				
Wt %	23.85	29.17	0	46.97
Mol %	32.41	30.49	0	37.11
Fraction 2[c]				
Wt %	0	47.98	6.33	45.70
Mol %	0	53.47[d]	8.04	38.49
Authentic 5,11,14,17-20:4				
Wt %	0	86.97	13.58	0
Mol %	0	84.81	15.19	0

[a]6A, n-hexanal; 5AE, methyl 5-oxo-pentanoate; 6AA, 1,6-hexanedial; 8AE, methyl 8-oxo-octanoate.
[b]Retention time on Silar 10C.
[c]Compositions given in text.
[d]Calculated percentages of the ozonolysis products from 5,11,14,17-20:4 in this fraction: 5AE 52.17 and 6AA 9.34.

The totals of 5,11- and 5,13-20:2 fatty acid methyl esters of acids from the total lipids of Echinoidea were as much as 2.6–10.0% in this study. These contents were relatively higher than in some other marine animals (12–14), where starfish *Asterias vulgaris* had the highest content at 5.6%, and most of the totals were less than 1%. However, the totals of 5,11- and 5,13-20:2 found in this study were rather lower than those reported for the Atlantic sea urchins (5-10%) (1). In all of the samples studied, the proportion of 5,11-20:2 was always higher than that of 5,13-20:2, similar to the results of the previ-

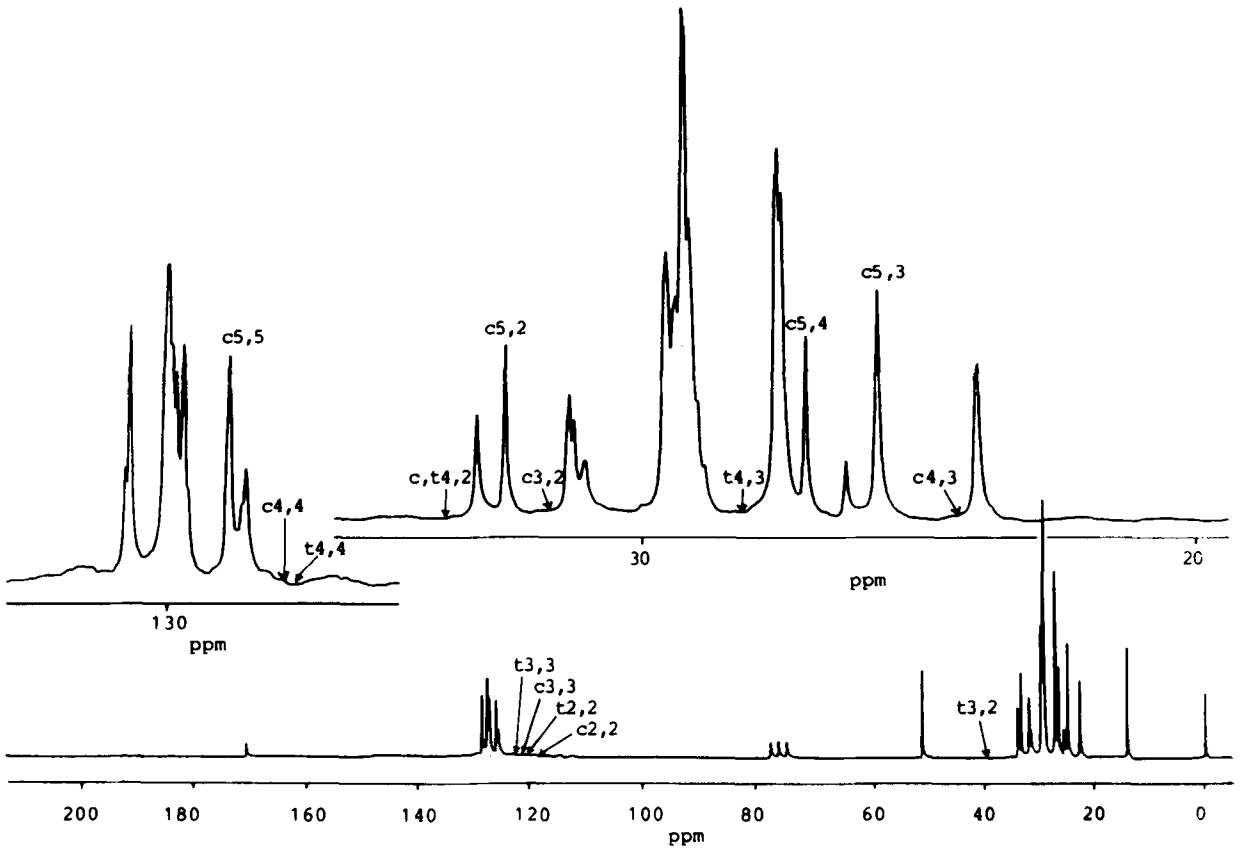

FIG. 2. ^{13}C-NMR spectrum of dienoate fraction from the lipids of salted gonads of sea urchins. c5,3: C_3 carbon of *cis*-5-olefinic acid; t3,2: C_2 carbon of *trans*-3-olefinic acid.

ous study (1).

The distribution of the 5-olefinic acids in triacylglycerols (TG) of *S. intermedius* was elucidated by Grignard hydrolysis and following GLC analysis in our previous study (15). These 5-olefinic acids distributed mainly in the 1(3)-position of TG.

Fatty acid compositions of TL. Samples 1–6 in Table 1 belong to the same animal order. Fatty acid compositions were similar in samples 1, 5 and 6, and in 3 and 4, in the contents of 5-20:1, 5,11- and 5,13-20:2, 20:4(n-6) and 20:5(n-3), respectively. Sample 2 showed a rather different composition. These samples were obtained from Hokkaido (3 and 4), Kanagawa (1,5 and 6) and Okinawa (2), respectively. The differences among the fatty acid compositions of these samples may be attributed to the environments and ecosystems of the respective habitats.

Samples 7–12 belong to different orders, and it is surprising that the fatty acid compositions of 7–12 showed individually different patterns. Samples 9 and 10 contained higher contents of 22:6(n-3). Since the proportions of 22:6(n-3) are usually very low among the fatty acids of marine algal lipids (16,17), the high content of this fatty acid in 9 and 10 suggests that it came from their diets, e.g., the carcasses of fish and other marine animals. Sample 9 was obtained at 740 m depth, where algae are not directly available to the sea urchins as part of the diet. In such a case, it is known that the diet includes the carcasses of marine animals (18). The animals belonging to the order Clypeasteroida (samples 11 and 12) live on sand bottoms, and their diets are generally detritus from marine animals and plants. Their specific fatty acid features (Table 4), notably the low (<3%) contents of 20:4(n-6), could be attributed to diets different from those of other sea urchins.

Fatty acid compositions of NL and PL. Fatty acid compositions of NL and PL are shown in Table 5. Fatty acids having a nonmethylene-interrupted (NMI) 5-olefinic bond [5-20:1 and 5,11-20:2] and those having a methylene-interrupted 5-olefinic bond with other olefinic bonds [20:4(n-6) and 20:5(n-3)] were generally rich in fatty acids from PL. On the contrary, monoenoic acids such as 18:1(n-9) and 18:1(n-7) and minor polyenoic components such as 18:2(n-6), 18:3(n-3), 18:4(n-3) and 22:6(n-3) were rich in fatty acids from NL. The total 5-olefinic acids in PL described above will have important roles as the constituents of lipids in membranes, as well as the source of supply for physiologically active components and biological energy. The NMI 5-olefinic bond increases the stability of the nearby ester linkage toward lipolytic hydrolysis, and the effect would increase stability of the membrane against the microbial lipases (12).

Branched and odd chain fatty acids. The fatty acids from samples 3, 4, 6, 7, 9, 10 and 11 in Table 1 contained high levels of iso and anteiso branched and odd chain fatty acids, totaling more than 4.7%, as shown by the data in Table 4. The high contents of the branched and odd chain acids in the neutral lipids of the gonads and visceras from a mud-feeding sea urchin, *S. franciscanus*,

were reported in a previous paper (19). High contents of the branched and odd chain acids were also found in other mud-feeding marine animals, such as the mullet *Mugil cephalus* (20,21), the smelt *Osmerus mordax* from a specific location (22) and the holothurian *Scotoplanes theeli* (23). The origin of these branched and odd chain acids in the mud-feeding marine animals is known to be bottom material taken into their diets. Both branched and odd chain acids in sediment and bottom material have been reported to be formed by the action of microorganisms (24). In this study, it is thought that the origin of the branched and odd chain acids in the lipids from the Echinoidea samples is also basically from their diets.

Origin of the unusual 5-olefinic fatty acids. Several explanations can be put forward to account for the 5-olefinic bond in the fatty acids of Echinoidea. These are in situ production by a 5-desaturase on preexisting fatty acids, absorption from lipids of food such as algae and sediment, assimilation from lipids of microorganisms normal in Echinoidea digestive system or absorption through the skin from seawater or epiflora. However, only trace amounts of 5-olefinic acids were found in most algae (16,17), with exceptions being *Cladophora rupestris* and *Ascophyllum nodosum* (25,26). Particularly, 5-20:1 in the major 5-olefinic acids of Echinoidea has not been reported among the constituents of algal lipids. The occurrence of the 5-olefinic acids has not been reported in marine microorganisms or in seawater. Therefore, the most plausible explanation for the origin of the 5-olefinic acids is that Echinoidea have a 5-desaturase. It is suggested that the biochemical formation of 5-20:1 from 20:0, 5,11-20:2 from 11-20:1, 5,13-20:2 from 13-20:1, 5,11,14-20:3 from 11,14-20:2 and 5,11,14,17-20:4 from 11,14,17-20:3 is by a 5-desaturase, as suggested for the sea urchin *S. droebachiensis* (1). The acyl group in seed oil of meadowfoam *Limnanthes alba* contains about 60% 5-20:1 with small amounts of 5-22:1, 5-18:1 and 5,13-22:1 as 5-olefinic acids, and 5-desaturation of fatty acids from oilseeds was demonstrated by incubation of ^{14}C-labeled substrates with developing seed slices and with a cell-free homogenate of meadowfoam (27,28). The unusual 5-olefinic acids in the Echinoidea can be formed in a similar manner by a 5-desaturase. An analogous desaturation has been suggested for the formation of the unusual 5-olefinic acids and the acids derived from C_2 elongation (7-olefinic acids) in gymnospermae seeds (4), and some molluscs (12, 29 and 30). The longer chain 5-olefinic fatty acids have also been found in some sponges, and formation by 5-desaturase has also been suggested (31,32) in these cases. However, the occurrence of the 5-monoenoic fatty acids in appreciable amounts has not yet been reported in lipids of other marine organisms. The facts suggest that the remarkable features of the 5-

TABLE 3

Comparison of Mass Spectra of 1,6-Hexanedial from Ozonolysis with a Reference Sample

m/e	Intensity percent[a]		Relation with M[b]
	Ozonolysis products	Reference	
113	3.0	1.3	M-1
96	57.7	42.7	M-18
95	22.8	18.6	M-18-1
85	2.6	2.8	M-29
84	1.8	3.7	M-29-1
78	1.6	0.5	M-18-18
70	65.5	63.7	M-44
69	5.2	4.0	M-44-1
68	18.3	18.9	M-46
67	100.0	100.0	M-18-29
57	45.8	36.5	M-57
56	2.7	1.3	M-29-29
44	4.3	2.7	M-70

[a]Percentages relative to the intensity of the base peak m/e 67.
[b]See text for the assignment. M = m/e of molecular ion 114.

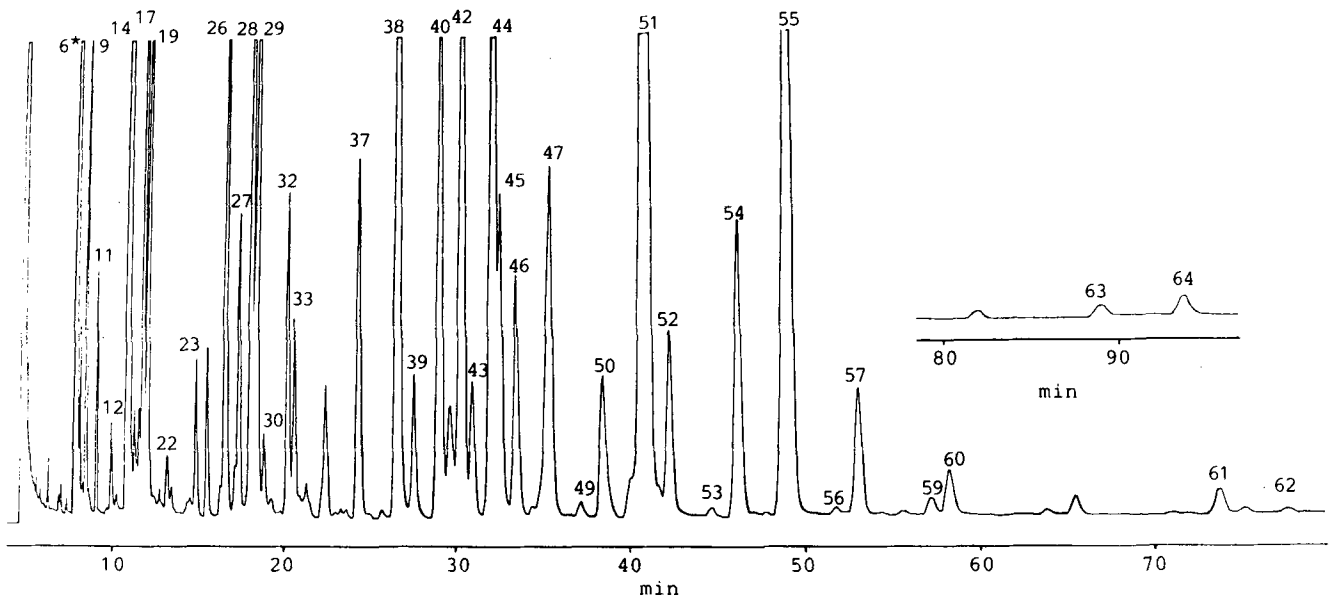

FIG. 3. Gas chromatogram of fatty acid methyl esters of total lipids from *Pseudocentrotus depressus*. *, See Table 4.

TABLE 4

Fatty Acid Compositions of the Total Lipids from Echinoidea (wt%)

No.	Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
1	*iso*-13:0	0.01	–	0.01	0.01	–	–	0.01	–	0.11	–	0.13	–
2	*anteiso*-13:0	–	–	0.01	–	–	–	0.01	–	–	–	–	–
3	13:0	0.31	0.28	0.03	0.01	0.37	0.02	0.08	0.06	1.58	0.09	5.52	–
4	*iso*-14:0	0.03	0.24	0.23	–	0.04	0.02	0.10	0.13	0.26	0.60	0.24	–
5	*anteiso*-14:0	–	–	–	0.03	–	–	0.02	–	–	–	–	–
6	14:0	15.15	9.31	4.61	4.25	8.40	19.69	8.17	15.75	3.90	4.43	2.78	2.62
7	14:1 (n-7)	0.05	0.26	–	0.13	0.04	0.05	–	–	0.09	–	0.46	0.01
8	14:1 (n-5)	1.14	0.83	–	–	0.64	–	–	0.70	1.56	2.23	2.42	–
9	*iso*-15:0	0.02	–	0.47	0.55	0.01	0.93	0.71	–	–	–	0.30	–
10	*anteiso*-15:0	0.07	–	0.20	0.37	0.09	0.05	0.88	–	0.80	–	0.70	0.21
11	15:0	0.38	1.73	0.33	0.96	0.45	0.32	1.01	1.07	1.13	2.19	1.96	0.44
12	*iso*-16:0	0.28	0.21	0.12	0.52	0.36	0.16	0.78	0.73	0.76	1.42	0.44	0.07
13	*anteiso*-16:0	–	–	0.12	0.42	–	–	0.47	–	–	–	–	–
14	16:0	16.42	18.39	14.45	9.84	16.30	16.42	12.25	20.81	10.81	15.53	18.87	14.96
15	16:1 5	0.20	0.18	0.17	1.73	0.36	0.18	1.74	0.13	0.81	0.53	–	0.15
16	16:1 (n-9)	0.20	0.70	0.47	0.48	0.26	0.16	–	0.15	0.98	0.67	2.57	14.11
17	16:1 (n-7)	1.60	3.92	6.04	1.99	2.35	2.21	5.06	3.25	4.47	9.05	3.60	–
18	16:1 (n-5)	5.02	0.60	–	–	1.39	–	–	0.77	1.97	2.08	0.33	0.05
19	*iso*-17:0	0.25	–	2.28	2.59	0.21	2.78	0.83	–	0.58	–	0.37	–
20	*anteiso*-17:0	0.04	–	0.06	0.41	0.09	0.01	1.00	–	0.48	–	0.64	–
21	16:2 (n-6)	–	0.46	0.23	0.23	0.02	–	0.08	0.13	0.16	0.34	0.11	0.29
22	17:0	0.27	0.43	0.30	1.18	0.31	0.11	1.05	1.29	1.19	1.03	1.98	0.37
23	*iso*-18:0	–	–	0.12	0.40	0.26	0.37	1.18	–	0.07	–	0.17	–
24	*anteiso*-18:0	–	–	–	0.17	–	–	0.06	–	–	–	–	–
25	16:4 (n-3)	3.69	2.16	0.59	0.43	2.27	0.06	–	0.22	1.10	0.11	1.33	1.30
26	18:0	3.57	4.24	2.16	2.61	3.14	2.09	3.22	3.43	4.46	3.35	10.22	3.72
27	18:1 5	0.50	0.16	1.01	1.00	0.58	0.77	0.98	1.20	1.79	1.27	0.83	0.45
28	18:1 (n-9)	0.65	0.59	2.85	0.98	2.03	2.53	6.23	6.26	2.59	1.41	6.27	1.64
29	18:1 (n-7)	4.43	2.75	3.01	3.28	3.56	2.77	6.89	3.27	5.43	6.11	4.68	3.88
30	18:1 (n-5)	0.36	–	0.29	–	0.12	0.23	0.37	0.26	0.49	0.64	0.16	0.04
31	*anteiso*-19:0	–	–	0.10	0.17	–	–	0.36	–	–	–	–	–
32	18:2 (n-9)	1.07	0.51	1.03	0.32	0.58	0.90	0.84	0.17	0.16	0.60	–	–
33	18:2 (n-6)	0.37	1.75	1.27	0.44	0.82	0.58	1.09	0.84	1.00	0.79	1.18	0.83
34	19:0	0.38	0.14	0.34	0.95	0.23	0.16	0.38	0.31	0.89	0.74	0.61	0.60
35	18:3 (n-6)	0.34	0.95	–	–	0.28	–	0.34	0.28	1.37	–	0.77	0.49
36	*iso*-20:0	–	–	–	–	–	–	0.35	–	–	–	–	–
37	18:3 (n-3)	0.43	3.47	0.82	0.30	1.90	1.23	1.15	0.78	0.08	0.16	0.19	0.36
38	18:4 (n-3)	1.09	3.83	0.93	0.33	4.18	4.27	0.82	1.54	0.26	0.29	0.38	1.26
39	20:0	0.85	0.19	0.46	0.59	0.78	0.60	0.89	0.77	0.75	0.67	1.34	0.74
40	20:1 5	3.75	2.10	7.76	8.25	3.84	2.57	4.05	3.67	6.85	5.32	4.53	5.22
41	20:1 (n-11)	2.14	0.29	0.94	1.37	0.96	0.58	2.09	1.77	0.65	0.58	1.60	0.10
42	20:1 (n-9)	2.17	0.51	2.53	2.21	2.98	3.76	1.52	2.76	0.52	0.69	1.62	4.17
43	20:1 (n-7)	0.83	0.34	0.81	1.28	0.81	0.57	0.98	1.00	0.39	0.49	0.86	3.60
44	20:2 5,11	5.17	2.31	6.69	7.23	4.59	4.97	3.04	3.32	3.10	4.14	2.17	3.72
45	20:2 5,13	1.34	0.27	3.27	2.47	1.56	1.25	0.80	0.67	1.45	0.91	0.63	2.80
46	20:2 (n-9)	0.20	–	0.06	–	0.05	1.14	0.33	0.12	–	0.08	–	0.04
47	20:2 (n-6)	2.67	1.15	2.83	1.98	1.46	2.17	0.82	1.01	0.94	0.34	0.40	1.10
48	20:3 (n-9)	–	1.50	–	–	–	–	0.87	0.88	–	0.50	–	–
49	20:3 5,11,14	0.08	0.35	0.27	0.40	0.25	0.08	0.26	0.06	0.43	0.24	0.15	0.18
50	20:3 (n-6)	0.79	0.85	0.80	0.25	1.03	0.85	0.59	0.41	0.19	0.20	0.89	0.28
51	20:4 (n-6)	10.53	15.22	15.44	19.21	10.80	10.76	8.50	7.77	15.12	8.00	3.16	2.93
52	20:3 (n-3)	0.79	–	0.56	0.73	2.01	1.08	0.48	0.07	0.16	0.04	–	1.91
53	20:4 5,11,14,17	0.09	0.53	0.01	0.06	0.21	0.06	0.01	0.02	0.06	0.03	0.08	0.02
54	20:4 (n-3)	0.80	1.80	–	–	1.95	1.79	0.25	0.24	–	0.24	0.11	0.10
55	20:5 (n-3)	6.21	12.65	6.54	7.83	9.91	6.54	11.86	6.53	10.31	13.82	2.29	18.26
56	22:1 (n-11)	–	–	–	–	0.19	0.06	0.47	0.19	0.34	0.21	0.66	0.86
57	22:1 (n-9)	1.87	0.27	2.01	1.19	1.77	0.87	1.55	0.96	0.30	0.41	2.18	2.99
58	22:1 (n-7)	–	–	–	0.02	0.09	0.02	0.17	0.44	0.28	0.07	0.93	0.47
59	22:2 7,13	0.13	–	0.46	0.51	0.14	0.13	0.23	0.29	0.12	0.09	0.45	0.01
60	22:2 7,15	0.25	–	2.13	2.39	0.71	0.34	1.14	0.14	0.99	0.49	0.21	0.38
61	22:4 (n-6)	0.40	0.20	0.23	0.39	0.51	0.24	–	0.29	0.58	0.27	1.73	0.75
62	22:5 (n-6)	–	–	0.11	0.39	0.29	0.05	0.07	0.36	1.06	0.38	0.36	–
63	22:5 (n-3)	–	0.83	0.75	0.56	0.26	0.15	0.07	0.29	0.55	0.58	0.25	0.06
64	22:6 (n-3)	0.64	0.55	0.69	2.59	1.20	0.26	0.46	2.45	3.55	5.58	1.96	1.23
65	24:1 (n-9)	–	–	–	0.03	–	–	–	0.02	–	–	1.25	0.22

TABLE 5

Fatty Acid Compositions of the Neutral and Polar Lipids from Echinoidea (wt%)

No.	Fatty acid	2 NL	2 PL	3 NL	3 PL	4 NL	4 PL	7 NL	7 PL	8 NL	8 PL	10 NL	10 PL
1	*iso*-13:0	–	–	0.01	–	0.03	0.03	0.01	0.01	–	–	–	–
2	*anteiso*-13:0	–	–	0.01	0.01	0.02	0.06	0.02	0.02	–	–	–	–
3	13:0	0.30	0.14	–	–	–	0.11	0.11	0.02	0.08	–	–	0.07
4	*iso*-14:0	0.32	0.07	0.09	0.09	0.16	0.13	0.14	0.06	0.19	0.08	0.18	0.46
5	*anteiso*-14:0	–	–	0.01	0.02	0.03	0.03	0.02	–	–	–	–	–
6	14:0	8.84	8.88	6.39	3.15	4.23	4.11	11.93	0.95	20.60	4.59	5.08	3.85
7	14:1(n-7)	0.36	0.06	0.19	0.04	0.27	0.06	–	–	–	–	–	–
8	14:1(n-5)	0.91	0.29	–	–	–	–	–	–	0.91	0.31	0.83	2.19
9	*iso*-15:0	–	–	1.14	0.19	0.92	0.36	0.98	0.13	–	–	–	–
10	*anteiso*-15:0	–	–	0.24	0.17	0.51	0.27	1.21	0.12	–	–	–	–
11	15:0	2.11	1.26	0.44	0.24	1.90	0.67	1.36	0.25	1.54	0.66	1.24	2.19
12	*iso*-16:0	0.28	0.04	0.21	0.08	1.01	0.27	0.67	0.87	0.69	0.16	0.50	1.29
13	*anteiso*-16:0	–	–	0.17	0.01	0.35	0.06	0.46	0.45	–	–	–	–
14	16:0	15.52	24.43	19.04	10.09	11.95	9.01	14.50	9.42	18.71	29.99	18.18	12.67
15	16:1 5	0.10	0.10	0.19	0.09	1.49	0.42	1.83	1.55	0.34	0.04	0.50	0.59
16	16:1(n-9)	1.22	0.12	0.59	0.33	0.55	0.41	–	–	0.20	0.14	0.30	0.73
17	16:1(n-7)	4.87	0.65	11.64	2.51	2.53	1.50	6.63	0.93	4.37	1.94	4.98	9.76
18	16:1(n-5)	0.68	0.17	–	–	–	–	–	–	1.00	0.32	1.23	2.13
19	*iso*-17:0	–	–	3.27	1.10	2.69	1.51	0.86	0.84	–	–	–	–
20	*anteiso*-17:0	–	–	0.11	0.07	0.51	0.16	0.95	0.63	–	–	–	–
21	16:2(n-6)	0.78	0.03	0.26	0.04	0.29	0.04	0.05	–	0.16	0.06	0.06	0.40
22	17:0	0.53	0.52	0.31	0.47	1.28	1.84	1.27	0.93	1.38	1.91	1.13	1.07
23	*iso*-18:0	–	–	0.07	0.14	0.89	0.18	0.59	3.02	–	–	–	–
24	*anteiso*-18:0	–	–	0.01	0.01	0.12	0.04	0.04	0.09	–	–	–	–
25	16:4(n-3)	2.91	0.26	0.54	0.06	0.28	0.10	–	–	0.14	0.17	0.88	0.06
26	18:0	3.01	6.70	1.72	2.20	2.89	1.51	3.05	5.15	2.95	5.11	3.40	3.87
27	18:1 5	0.15	0.11	0.71	1.33	0.71	1.09	0.72	1.01	1.13	0.66	1.21	1.57
28	18:1(n-9)	0.91	0.29	5.37	1.15	1.46	0.76	7.75	1.76	7.03	1.86	3.56	1.48
29	18:1(n-7)	3.64	1.05	4.08	2.43	4.00	2.88	7.69	3.50	3.93	1.14	7.26	4.76
30	18:1(n-5)	0.37	0.05	0.30	0.25	0.51	0.34	0.37	0.02	0.02	0.20	0.70	0.32
31	*anteiso*-19:0	–	–	0.03	0.03	0.19	0.08	0.31	0.11	–	–	–	–
32	18:2(n-9)	0.61	0.07	1.71	0.69	0.35	0.20	0.90	0.16	0.10	–	0.06	0.26
33	18:2(n-6)	2.04	0.40	2.15	0.47	0.61	0.24	1.35	0.27	0.95	0.33	0.84	0.57
34	19:0	0.32	0.73	0.26	0.57	0.82	0.67	0.35	0.47	0.28	0.66	0.27	0.64
35	18:3(n-6)	1.12	0.16	–	–	–	–	0.44	–	0.36	0.10	–	–
36	*iso*-20:0	–	–	–	0.02	–	0.10	0.40	0.09	–	–	–	–
37	18:3(n-3)	3.86	0.82	1.27	0.29	0.67	0.16	1.41	0.43	0.97	0.37	0.33	0.16
38	18:4(n-3)	4.78	0.36	0.26	0.04	0.61	0.12	1.20	0.18	1.81	0.36	0.26	0.32
39	20:0	0.41	0.64	0.55	0.54	0.98	0.40	1.08	0.70	0.62	1.04	0.91	0.77
40	20:1 5	1.73	2.90	3.38	13.26	10.80	7.99	2.87	8.21	2.59	6.13	5.75	5.33
41	20:1(n-11)	0.43	0.75	0.93	–	2.51	1.05	2.33	1.47	1.36	3.76	1.24	0.64
42	20:1(n-9)	0.88	0.77	3.26	2.24	3.48	1.75	1.64	0.91	3.03	2.11	1.45	0.81
43	20:1(n-7)	0.55	0.39	1.07	0.67	2.12	0.93	1.25	0.76	0.95	0.68	0.77	0.57
44	20:2 5,11	2.04	2.92	5.64	8.86	4.78	9.33	1.88	4.93	3.23	3.91	3.06	5.05
45	20:2 5,13	0.37	0.31	2.98	3.30	2.13	2.89	0.76	0.96	0.48	0.31	0.71	1.12
46	20:2(n-9)	0.06	0.05	0.13	0.10	0.11	0.05	0.25	0.07	0.12	–	0.04	0.08
47	20:2(n-6)	1.00	1.08	2.07	3.92	2.29	1.75	0.91	1.39	0.80	1.02	–	0.44
48	20:3(n-9)	1.39	3.26	–	–	–	–	–	1.18	0.72	1.79	3.23	0.55
49	20:3 5,11,14	0.33	0.25	0.24	0.43	0.30	0.51	0.03	0.15	0.04	0.06	0.11	0.25
50	20:3(n-6)	0.94	0.65	1.11	–	0.51	0.18	0.58	0.23	0.31	0.56	0.15	0.21
51	20:4(n-6)	14.73	16.70	7.76	21.06	7.10	24.86	2.11	17.32	5.66	14.16	4.71	9.55
52	20:3(n-3)	–	0.04	0.59	0.91	1.01	0.73	0.26	0.62	0.04	0.06	0.04	0.07
53	20:4 5,11,14,17	0.95	0.19	0.08	0.09	0.12	0.06	0.01	0.01	0.01	0.01	0.02	0.05
54	20:4(n-3)	1.65	0.53	–	–	0.02	0.24	0.45	0.07	0.21	0.08	0.14	0.32
55	20:5(n-3)	9.99	18.12	4.14	8.43	3.82	9.38	5.53	21.03	4.93	7.18	12.78	16.07
56	22:1(n-11)	–	0.09	0.02	0.04	0.27	0.08	0.27	0.27	0.14	0.15	0.95	0.30
57	22:1(n-9)	0.51	0.79	0.06	2.14	2.04	1.41	1.94	1.77	0.91	1.00	0.92	0.55
58	22:1(n-7)	–	0.04	0.13	0.06	0.20	0.06	0.22	0.21	0.46	1.45	0.16	0.08
59	22:2 7,13	–	0.10	1.00	0.55	0.91	0.48	0.32	0.20	0.31	0.29	0.21	0.15
60	22:2 7,15	–	0.13	0.03	3.18	6.49	2.49	1.19	1.76	0.12	0.18	1.04	0.63
61	22:4(n-6)	0.25	0.41	0.02	0.24	0.16	0.32	0.21	0.12	0.34	0.35	0.18	0.32
62	22:5(n-6)	0.19	0.18	0.17	0.14	0.45	0.37	0.44	0.33	0.28	0.44	0.39	0.55
63	22:5(n-3)	0.64	0.50	0.99	0.71	0.66	0.57	0.70	0.30	0.34	0.30	0.67	0.84
64	22:6(n-3)	0.45	0.46	0.79	0.70	1.92	2.60	3.19	1.54	2.15	1.84	7.20	3.18
65	24:1(n-9)	–	–	0.06	–	–	–	–	–	–	–	0.23	0.09

desaturase in Echinoidea are a high activity which desaturates saturated fatty acids to the corresponding 5-monoenoic acids similarly to the meadowfoam seeds. ^{13}C-NMR analyses of fatty acid methyl esters from the sea urchin lipids did not show any occurrence of the 3-olefinic acids. Thus, it is hardly thought that the 5-olefinic acids are derived from C_2 elongation of the 3-olefinic acids formed by 3-desaturase.

Recently, fatty acid compositions for sea urchins and other marine invertebrates from the North Pacific have been reported (33). In that study, unusual 5- and 7-olefinic acids were not reported. It is believed that this is due to the low resolution of the packed column used in the GLC analysis. Open tubular GLC is a better tool for this purpose.

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REFERENCES

1. Takagi, T., Eaton, C.A., and Ackman, R.G. (1980) *Can. J. Fish. Aquat. Sci. 37*, 195-202.
2. Itabashi, Y., and Takagi, T. (1980) *Yukagaku 29*, 855-865.
3. Takagi, T., Kaneniwa, M., and Itabashi, Y. (1982) *Bull. Fac. Fish, Hokkaido Univ. 33*, 263-269.
4. Takagi, T., and Itabashi, Y. (1982) *Lipids 17*, 716-723.
5. Bligh, E.G., and Dyer, W.J. (1959) *Can. J. Biochem. Physiol. 37*, 911-917.
6. Kleiman, R., Spencer, G.F., Earle, F.R., and Wolff, I.A. (1969) *Lipids 4*, 135-141.
7. Ackman, R.G. (1964) *J. Gas Chromatogr. 2*, 173-179.
8. Kochi, M. (1976) *J. Shimonoseki Univ. Fish. 25* 83-153.
9. Kochi, M. (1976) *Bull. Jpn. Soc. Sci. Fish. 42*, 721-729.
10. Bus, J., Sies, I., and Lie Ken Jie, M.S.F. (1977) *Chem. Phys. Lipids 18*, 130-144.
11. Christie, W.W. (1982) *Lipid Analysis*, 2nd edn., pp. 79, Pergamon Press, Oxford.
12. Ackman, R.G., and Hooper, S.N. (1973) *Comp. Biochem. Physiol. 46B*, 153-165.
13. Paradis, M., and Ackman, R.G. (1977) *Lipids 12*, 170-176.
14. Paradis, M., and Ackman, R.G. (1975) *Lipids 10*, 12-16.
15. Takagi, T., Hayashi, K., and Itabashi, Y. (1981) *Proc. Jpn. Conf. Biochem. Lipids 23*, 254-257.
16. Ackman, R.G., and McLachan, J. (1977) *Proc. N. S. Inst. Sci. 28*, 47-64.
17. Johns, R.B., and Nicholes, P.D. (1979) *Phytochemistry 18*, 799-802.
18. Lawrence, J.M. (1975) *Oceanogr. Mar. Biol. Ann. Rev. 13*, 213-286.
19. Hayashi, K., and Takagi, T. (1977) *Bull. Fac. Fish. Hokkaido Univ. 28*, 40-46.
20. Sen, N., and Schlenk, H. (1964) *J. Am. Oil Chem. Soc. 41*, 241-248.
21. Deng, J.C., Orthoefer, F.T., Dennison, R.A., and Watson, M. (1976) *J. Food Sci. 41*, 1479-1483.
22. Addison, R.F., and Ackman, R.G. (1970) *Lipids 5*, 554-557.
23. Lewis, R.W. (1967) *J. Fish. Res. Board Can. 24*, 1101-1115.
24. Leo, R.F., and Parker, P.L. (1966) *Science 152*, 649-650.
25. Ratnayake, W.N., and Ackman, R.G. (1979) *Lipids 14*, 580-584.
26. Ackman, R.G. (1981) in *New Sources of Fats and Oils* (Pryde, E.H., Princen, L.H., and Mukherjee, K.D., eds.) pp. 189-220, American Oil Chemists' Society, Champaign , IL.
27. Pollard, M.R., and Stumpf, P.K. (1980) *Plant Physiol. 66*, 649-655.
28. Moreau, R.A., Pollard, M.R., and Stumpf, P.K. (1981) *Arch. Biochem. Biophys. 209*, 376-384.
29. Klingensmith, J.S. (1982) *Lipids 17*, 976-981.
30. Irazu, C.R., Pollerro, R.J., and Brenner, R.R. (1984) *Lipids 19*, 649-655.
31. Morales, R.W., and Litchfield, C. (1977) *Lipids 12*, 570-576.
32. Walkup, R.D., Jamieson, G.C., Ratcliff, M.R., and Djerassi, C. (1981) *Lipids 16*, 631-646.
33. Isay, S.V., and Busarova, N.G. (1984) *Comp. Biochem. Physiol. 77B*, 803-810.

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