Determination of the Identity of By-Products in the Industrial Production of Saturated Fatty Acids

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ABSTRACT: A technical product of saturated fatty acids has been analyzed to determine the structure of by-products found in the C_{20-22} fatty acids manufactured from fish oil. Short-path distillation of the weakly colored product provided a residue, which was extracted either by supercritical carbon dioxide or by acetone. Extracts and residues were analyzed by size-exclusion chromatography, supercritical fluid chromatography, and by gas chromatography, the latter combined with mass spectrometry and Fourier-transform infrared spectroscopy, and by nuclear magnetic resonance spectrometry. A series of homologs of fatty acid dimers was identified as lactone esters. Each homolog also contained several isomers with a varying number of carbon atoms in the two hydrocarbon chains of the dimers. Trimers, containing yellow components, were also present in small amounts, but the structure of the trimers has not been determined yet.

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KEY WORDS: Fatty acid by-products, fatty acid dimers, fish oils, lactone esters, supercritical fluid chromatography.

Saturated fatty acids for industrial use are manufactured from a variety of fats, including fish oils. The manufacturing process consists of refining, hydrogenation, hydrolysis, and fractional distillation. Fish oils contain (mainly unsaturated) fatty acids with 12–24 carbon atoms, but more narrow fractions can be selected by fractional distillation. In our study, the product contained saturated fatty acids mainly with 20 and 22 carbon atoms, together with minor amounts of by-products of unknown identity.

It is well known that unsaturated fatty acids may be oxidized to hydroperoxides (1) and that dimers and trimers may be formed, partially by Diels-Alder additions (2). Burkow and Henderson (3,4) found that autoxidation of fish oils led to the formation of polymers, but no structure information was reported.

Because the industrial hydrogenation processes are never 100% complete, the formation of higher-molecular-weight products likely occur before and during final distillation. A sign of the presence of unwanted by-products in fatty acids is a weak yellow color in an otherwise colorless product. The purpose of this investigation has been to obtain information of the structure of those by-products.

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EXPERIMENTAL PROCEDURES

Chemicals and samples. The samples were provided by Pronova Oleochemicals A/S (Sandefjord, Norway). Fatty acid standards came from Larodan AB (Malmoe, Sweden). Solvents of high-performance liquid chromatography (HPLC) grade came from Rathburn Chemicals Ltd. (Walkerburn, Scotland). The CO_2 was 99.998 grade from AGA Norgas (Oslo, Norway).

Instruments and methods. Molecular distillation (short-path distillation) of a technical product of C_{20+22} fatty acids was performed with a Leybold Heraus KDL-4 instrument (Leybold, Köln, Germany) at 150–175°C and $2 \cdot 10^{-3}$ bar. Supercritical-fluid extraction (SFE) with CO_2 was performed (at 45°C and pressures from 150 to 400 bar) on 0.4-g samples of the molecular distillation residue (MDR). The SFE instrumentation consisted of a homebuilt apparatus with an Isco μLC 500 pump, a Varian Aerograph gas chromatography (GC) oven, a 0.83-mL extraction chamber (Keystone, Bellefonte, PA) and a heated homemade stainless-steel restrictor, with an outside temperature of 180°C at a CO_2 flow of 0.4 mL/min. With 136 mL (liquid) of CO_2, 75% (by weight) of the sample was extracted. The extract was white, while the residue was weakly yellow.

Solvent extraction of the MDR was performed by treating 0.75 g of MDR with 3 mL acetone on an ultrasonic bath (Elma Transsonic T 420, Singen, Germany) for 5 min. After centrifugation (Piccolo 720; Heraus Christ, Herz, Switzerland) for 1 min, the solids were treated two times more with 1.5 mL acetone. The extracts were collected, concentrated to one-third of the volume, left for 12 h, and centrifuged. The acetone phase was yellow. The precipitate (fatty acids and dimers) was white.

Gel permeation chromatography (GPC) was performed with tetrahydrofuran, purified on basic active alumina (Merck, Darmstadt, Germany) on a Waters Styragel HR2 5 μm 100 A column (300 × 7.8 mm) at a mobile phase flow of 0.5 mL/min. The HPLC apparatus consisted of a Waters M 510 pump (Waters/Millipore Corp., MA), an HP 1040 A ul-

traviolet (UV) diode array detector (Hewlett-Packard Co., PA), and a Waters R 401 differential refractometer.

Supercritical fluid chromatography with flame-ionization detection (SFC–FID) was performed with CO_2 on a 10 m by 50 μm i.d. Dionex SB-Biphenyl-30 column (0.25 μm film thickness) from Lee Scientific (Salt Lake City, UT). The Carlo Erba 3000 SFC instrument (Carlo Erba, Milan, Italy) was equipped with a time-split injector and a flame ionization detector (at 350°C). SFC–mass spectrometry (SFC–MS) was performed on the same column on a Lee Scientific series 600 SFC instrument, hooked up to a JEOL JMS-DX 303 mass spectrometer (JEOL, Tokyo, Japan). The homemade fused-silica integral restrictor was heated to 190°C. The ion source temperature was 150°C. Isobutane (0.015 mtorr) was added for chemical ionization (CI).

GC–MS was performed on a 30 m × 0.32 mm Supelco SP 2100 column (Supelco, Bellefonte, PA) with helium on a Fisons GC 8000-Fisons VG Prospec instrument (Poole, United Kingdom). The temperature program was started at 150°C for 2 min, then 20°C/min. to 250°C and 5°C/min to 300°C. GC–FTIR was performed on a 25 m × 0.32 mm i.d. Supelco SPB5 column with N_2 on a Nicolet Magna 550 instrument, equipped with a Varian 3300 gas chromatograph (Palo Alto, CA). NMR was performed in $CDCl_3$ at 35°C on a Varian Gemini 200 MHz instrument.

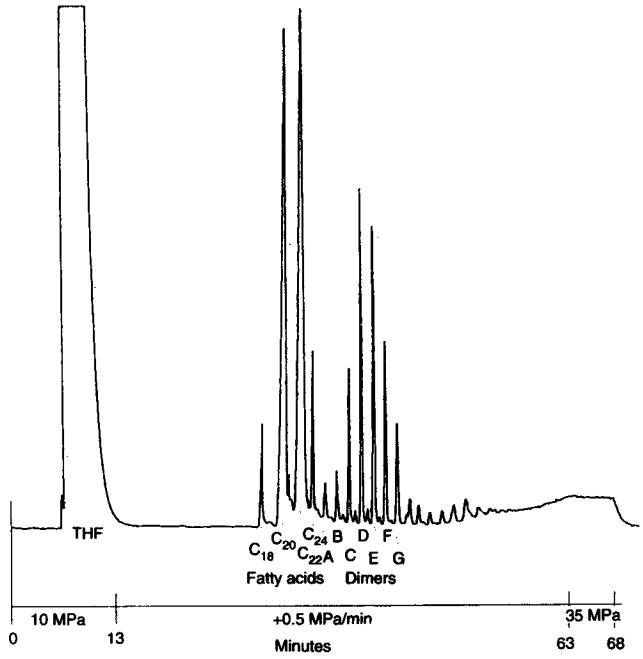


FIG. 1. Supercritical fluid chromatography with flame-ionization detection of molecular distillation residue of fatty acids product. The separation was performed at 100°C with the pressure program shown in the figure. THF, tetrahydrofuran.

RESULTS AND DISCUSSION

Analysis of the concentrate after molecular distillation. Molecular distillation of a weakly yellow-colored product of C_{20-22} fatty acids resulted in a residue with a stronger yellow color. The weight of the residue was reduced to 6.25% of the starting material. MS indicated the presence of compounds with molecular weights (MW) of at least two times that of the fatty acids, but the residue still contained fatty acids as the major components. GPC with refractive index detection showed two peaks: the first was assigned to a mixture of dimeric products, and the second (and the highest) consisted of the C_{20-22} fatty acids. A UV detector revealed the additional presence of a small but early eluting peak, which absorbed in the visible region at 380 nm. This peak was later assigned to products with MW approximately three times those of the fatty acids. Because SFC has been successful in separating lipids, the concentrate was analyzed by open tubular SFC with CO_2 and FID, which revealed a series of components with higher retention than the fatty acids (Fig. 1). The relative amounts of each peak were calculated (Table 1) by using the fatty acids as standards and assuming equal response factors. The combined dimers amounted to 41.9% of the MDR. Because the molecular distillation had resulted in a 16 times concentration, the actual concentration of the dimers in the product under investigation was 2.6%.

Analysis of purified concentrate. The concentrate was fractionated by two different methods: (i) Extraction by supercritical CO_2 resulted in an extract that contained fatty acids, dimers, and compounds with active oxygen (peroxides/hydroperoxides), which could be separated by GPC as shown in Figure 2A. The residue after extraction contained the major part of the colored components (Fig. 2B). Heating the colorless extract in inert atmosphere at 140°C for 3–12 h resulted in formation of a yellow material that absorbed at 380 nm. A peroxide test (5) indicated that the MDR concentrate contained approximately 0.1% organic peroxides. Based on size exclusion chromatography the yellow compounds, formed by heating, appeared similar to the original colored components in the residue, but no actual identification was obtained. Both extract and residue were analyzed by SFC–FID and SFC–MS, by CI as well as by charge exchange ionization. MW from 452 to 648 were found in a series of homologs of dimers (Table 1). The dimers were extracted with CO_2 together with the fatty acids.

TABLE 1
Amount of Each Dimer in the Molecular Distillation Residue (MDR)[a]

Dimer	Molecular weight	Weight %
A	452	1.8
B	480	2.7
C	508	5.3
D	536	10.9
E	564	9.9
F	592	6.5
G	620	3.7
H	648	1.1

[a]Based on supercritical fluid chromatography–flame-ionization detection. The values are calculated from three replicates with relative standard deviation of 2–9%.

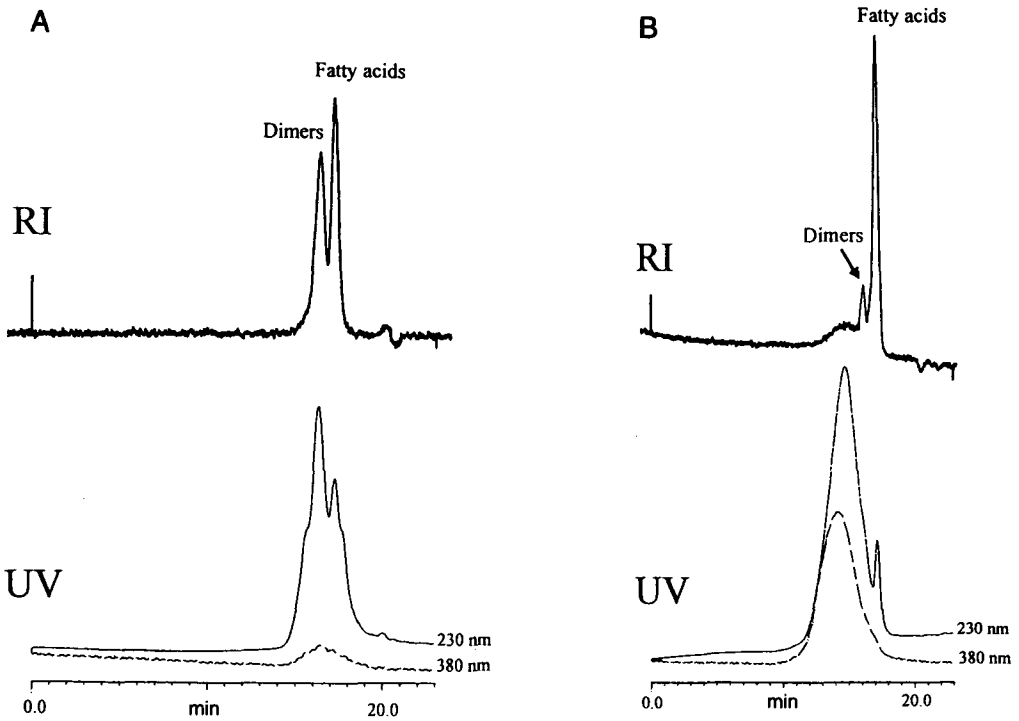


FIG. 2. Gel permeation chromatography with refractive index (RI) and ultraviolet (UV) detection of the extract (A) and the residue (B) after supercritical fluid extraction of the molecular distillation residue product.

(ii) Extraction and reprecipitation of the concentrate in acetone gave an extract that contained dimers, peroxides, and colored compounds and some fatty acids (Fig. 3B), while the bulk of the fatty acids and the dimers were precipitated (Fig. 3A). This precipitate was easy to work up in larger quantities and was used for nuclear magnetic resonance (NMR) studies. Proton NMR demonstrated (Fig. 4) a triplet at 4.04 ppm, not present in the spectrum of the pure fatty acid stan-

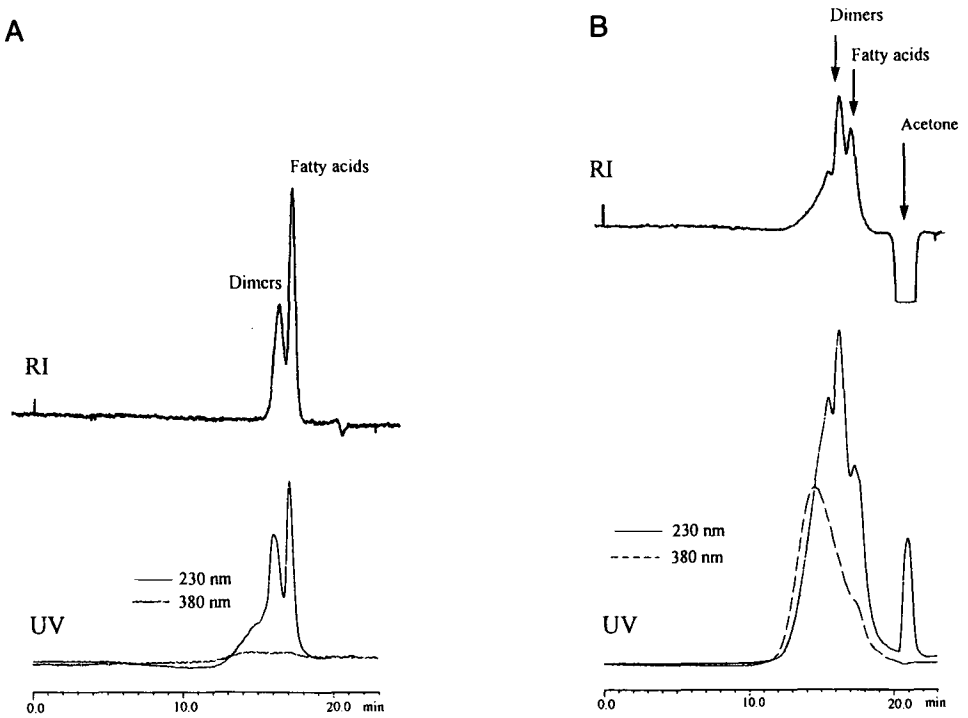


FIG. 3. Gel permeation chromatography with RI and UV detection of the residue (A) and the extract (B) after acetone extraction of the molecular distillation residue product. See Figure 2 for abbreviations.

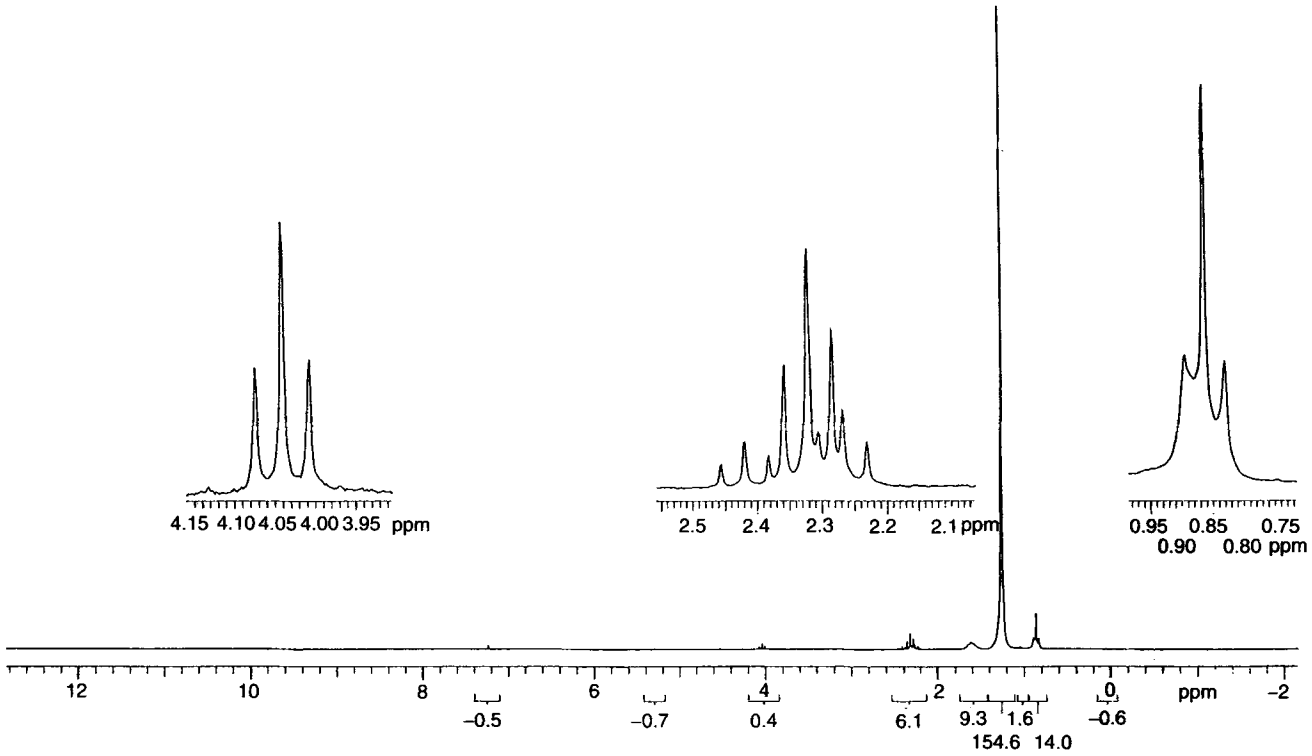


FIG. 4. Proton nuclear magnetic resonance in $CDCl_3$ of the residue after acetone extraction of the molecular distillation residue product.

dards, which was assigned to protons alpha to an oxygen atom (6). Methylene protons alpha to CHOCOR were found at 2.42 ppm, alpha to COOR at 2.27 ppm, alpha to CHO at 1.6 ppm, alpha to carbon only at 1.3 ppm, and finally, the methyl groups at 0.9 ppm. ^{13}C spectra (Fig. 5) showed two different carbonyl carbon atoms at 173 and 169 ppm and two different carbon atoms alpha to oxygen around 65 ppm. In addition, the spectra contained the fatty acid bands from this mixture of dimers and acids. Ether-linked and epoxide-linked carbon atoms were not found.

Structure of the dimers. Positive and negative ion MS, after different types of ionization, indicated the absence of free carboxylic groups in the dimer fraction. Because high-resolution separation techniques were needed due to the quite complicated mass spectra of the extracts, and because GC now appeared to be possible, GC–MS and GC–FTIR were attempted to obtain more structure information.

GC–MS (Fig. 6) resulted in higher resolution of the dimers than SFC–MS. As with SFC–MS, a series of homologs were evident with *m/z* differences of 28 of the highest *m/z* values. However, SFC constituted the best representation of the distribution of homologs, because the higher homologs more or less disappeared in GC.

The highest *m/z* value in the CI mass spectrum of each peak was ascribed to the $(M + 1)^+$ ion. The corresponding M^+ ions were present in the GC–electron ionization (EI) mass spectra. The maximum *m/z* value in the mass spectrum of a few small peaks between the major peaks differed by *m/z* 14 compared to that of the major peaks. The isobutane CI and the EI mass spectra of each peak contained ions that could not all be fragment ions from a single component. For dimer D, shown as an example (Fig. 7A and 7B), the fragments 341, 313, 285, 257, and 229 could not be explained by one compound. The apparent conclusion was that each peak contained isomeric dimers with equal number of carbon atoms but put together in different chainlengths, without affecting the volatility and the retention in GC. The EI mass spectra resembled the mass spectra of long-chain fatty acid esters (RCOOR'). The ion at *m/z* 257 in Figure 7 is most likely an $(RCOOH + H)^+$ ion, which is a dominating ion for fatty acid esters. Elimination of RCOOH from a long-chain fatty acid ester results in ions of the $(R'H)^+$ type; in this mass spectrum this corresponds to the ion at *m/z* 280. Other combinations that give $M^+ = 536$ are 341/196, 313/224, 285/252, and 229/308, for the pair $(RCOOH + H)^+$ and $(R'H)^+$. The ions at the lower m/z values are typical of the fragmentation for the R part of long-chain saturated fatty acid esters. No fragment ion was found that could give structural information of the R' group.

GC–FTIR of the SFE extract confirmed the presence of ester/lactone carbonyl (at around 1750 cm^{-1}). The FTIR spectra also confirmed the absence of free acids and, equally important, demonstrated the absence of hydroxyl groups and C–C double bonds. A typical example of the FTIR spectra of the dimers A to F (which were all similar), compared to fatty acids, is shown in Figure 8. The bands at 2930 and 2860

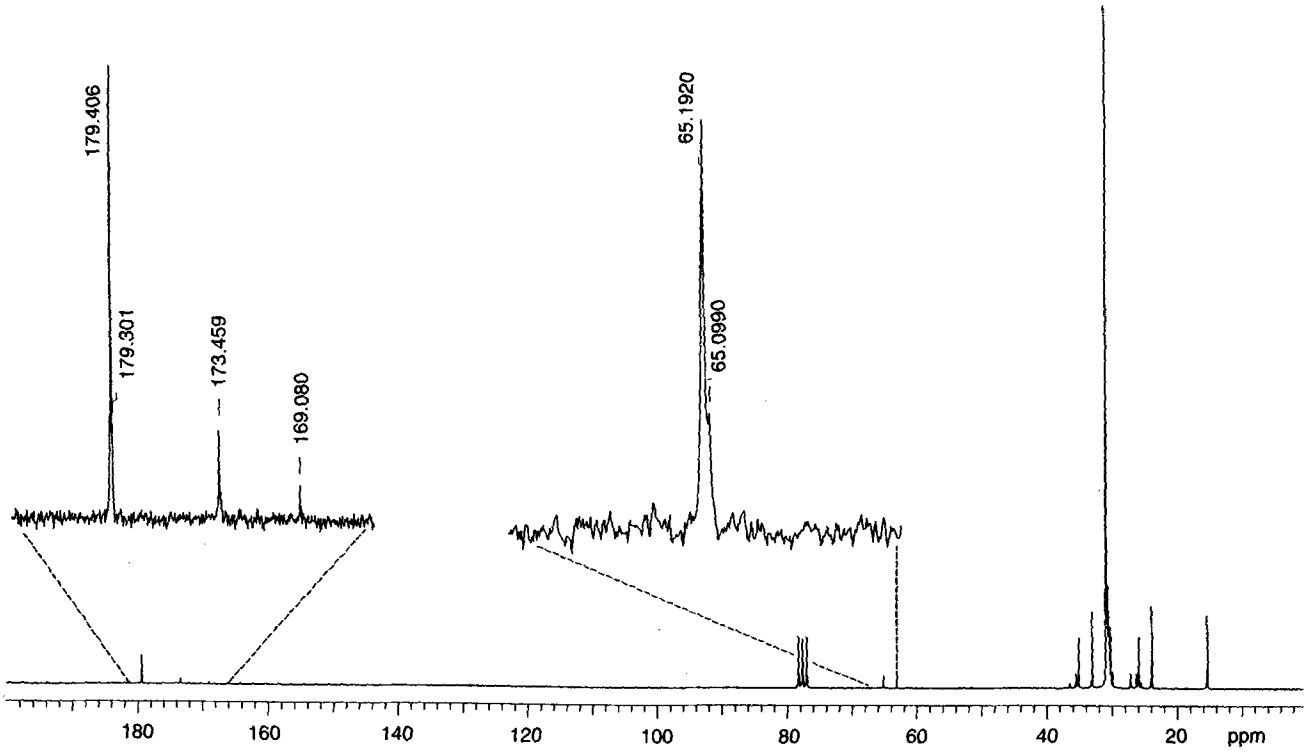


FIG. 5. ^{13}C nuclear magnetic resonance of the residue after acetone extraction of the molecular distillation residue product.

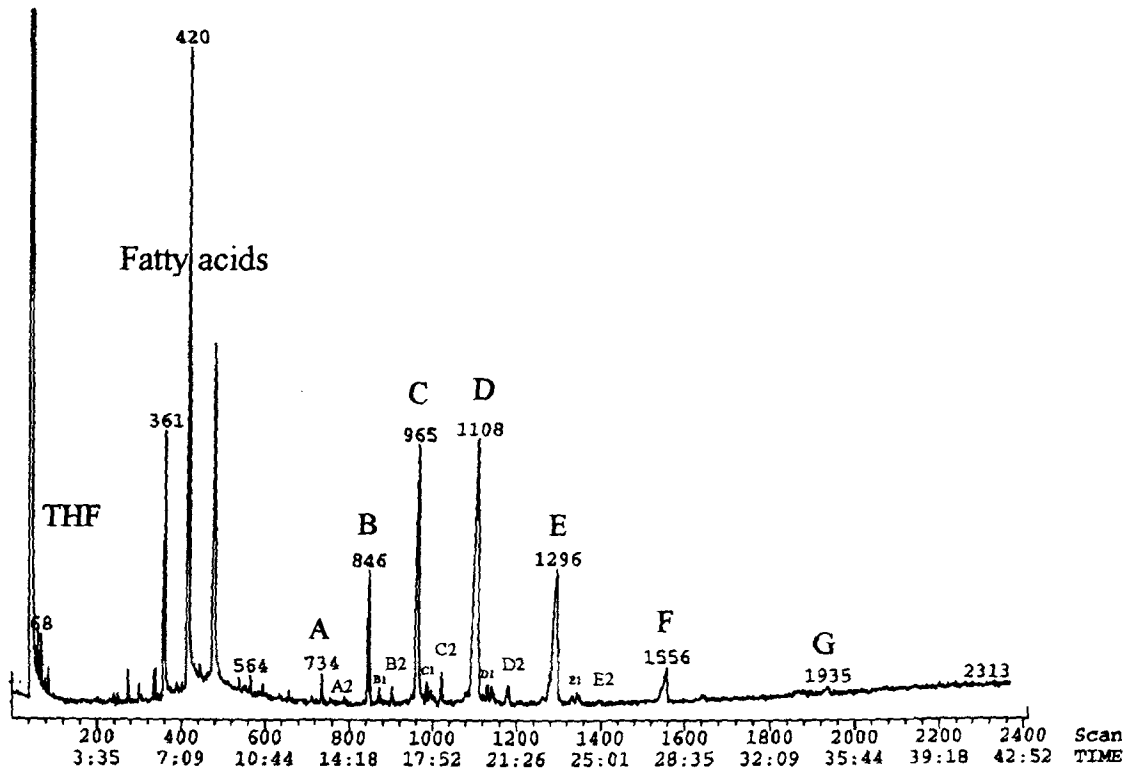


FIG. 6. Gas chromatography–mass spectrometry of the extract after supercritical fluid extraction of the molecular distillation residue product.

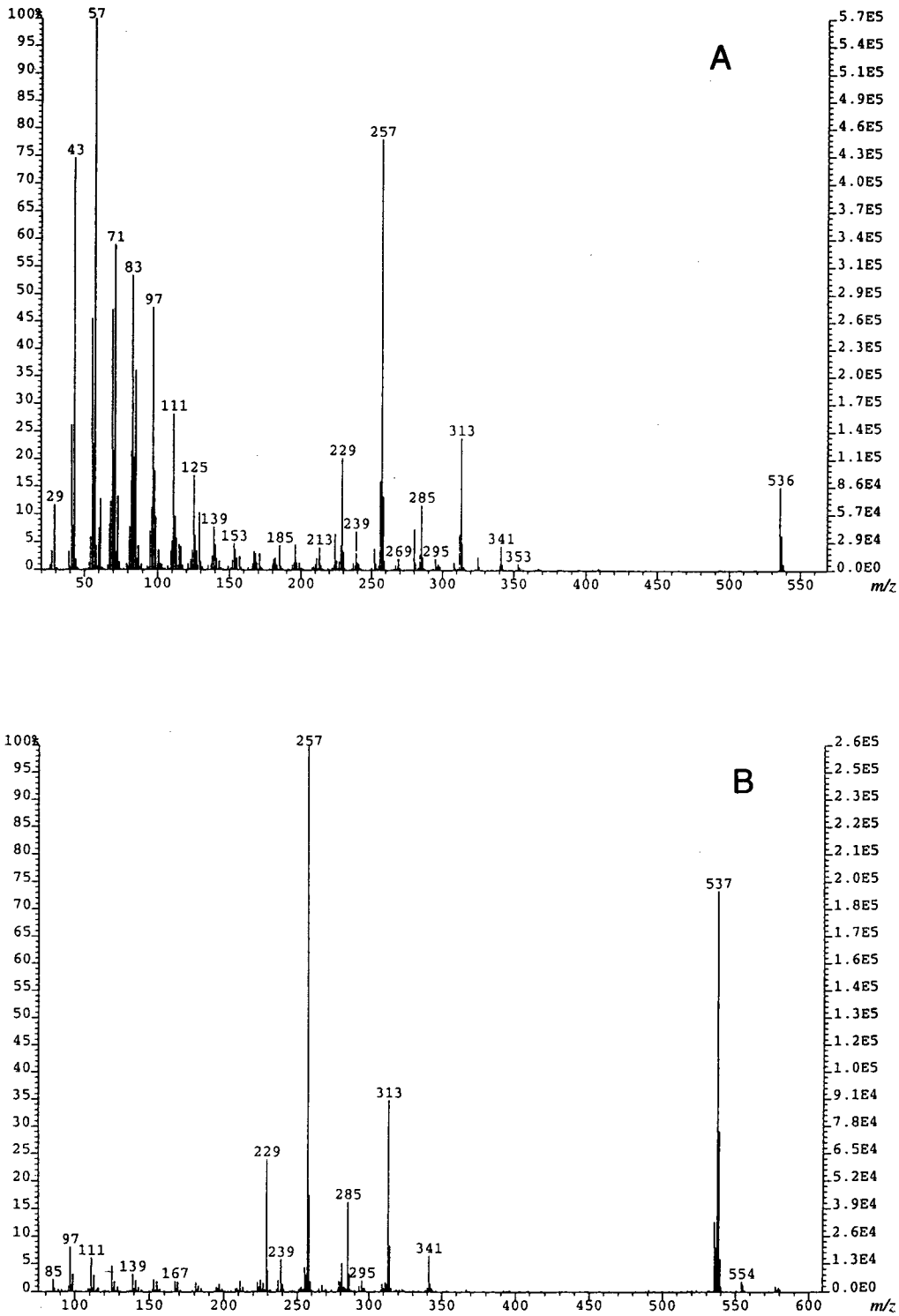


FIG. 7. Gas chromatography–mass spectra of dimer D with electron ionization (A) and chemical ionization (B).

cm^{-1} are due to C–H stretch, at 1460 and 1350 to C–H bending, and the bands at 1240 and 1160 cm^{-1}can be assigned to C–O stretching.

In theory, dimeric fatty acids can be linked by peroxide bonds, by ether bonds, by ester bonds, or by carbon-carbon bonds in a linear or in a cyclic structure. In the absence of ether and peroxide linkages (NMR data) and in the absence of free acids (MS and IR data), the dimers had to be compounded by a combination of an ester bond and a lactone ring (Scheme 1) to fit the MS data. The ester chain contained between 14 and 22 carbon atoms, while the lactone chain varied between 12 and 20 in the dimers from even-numbered fatty

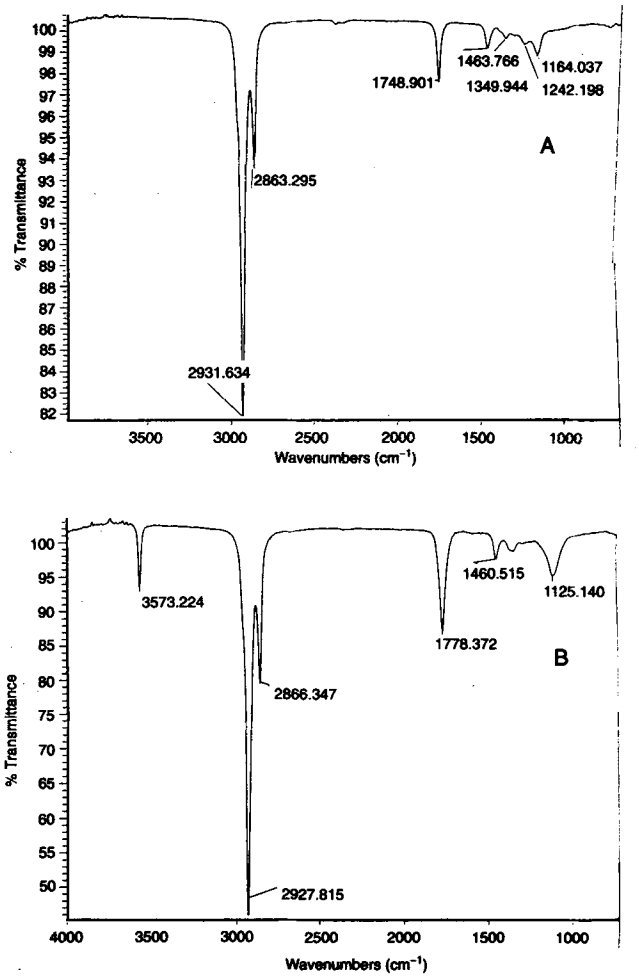
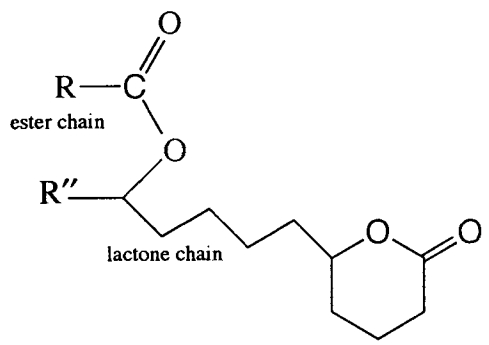


FIG. 8. Gas chromatography–Fourier transform infrared of dimer D (A) and of *n*-C_{22} fatty acid (B).

acids. Based on the patterns of the GC–MS EI and CI mass spectra, the relative amount of isomers with varying length of the carbon chain in the ester part (R) and in the lactone part was determined for each homolog dimer (Table 2).

The small peaks between the major ones were composed of ester lactones from fatty acids with uneven numbers of carbon atoms, which may have been present in minor amounts in the marine oils or which may have been formed by breakdown of larger acids (Table 3).



SCHEME 1

Formation of ester lactones. Lactones are cyclic esters formed from hydroxy acids. During the hydrolysis of triglycerides, hydroxy acids can be formed directly by acid-catalyzed hydroxylation of unsaturated fatty acids or by decomposition of initially formed hydroxyperoxides. Heating to 250°C during distillation can obviously lead to ester formation between one fatty acid and a dihydroxy acid, followed by lactonization. Estolides, which are esters between a hydroxy fatty acid and another fatty acid, can be formed in a batch reactor (7) but are also found naturally in oils that contain large amounts of hydroxy fatty acids (8).

Depending on the length of the fatty acid chain and the position of the hydroxyl groups, a number of isomers can be produced, with different length of the chain in the lactone part vs. the ester part but otherwise with similar properties. The distribution of isomers within one homolog dimer was largely representative for the availability of saturated fatty acids for ester formation. With a large preponderance of C_{20} and C_{22} fatty acids, these acids were found in the ester part for all possible combinations (dimers D, E, F, and G). Dimers A and B are made from the less available smaller fatty acids. The length of the hydrocarbon chain in the lactone part is a func-

TABLE 2
Number of Carbon Atoms in the Ester Chain (R) and the Lactone Chain and Relative Amounts of Isomers in Each Dimer[a]

		Number of carbon atoms		
Dimer	MW	Ester chain	Lactone chain	Relative amount (%)
A	452	14	14	61
A	452	16	12	39
B	480	14	16	11
B	480	16	14	74
B	480	18	12	15
C	508	14	18	37
C	508	16	16	20
C	508	18	14	33
C	508	20	12	10
D	536	14	20	13
D	536	16	18	55
D	536	18	16	9
D	536	20	14	19
D	536	22	12	4
E	564	16	20	38
E	564	18	18	38
E	564	20	16	9
E	564	22	14	15
F	592	18	20	33
F	592	20	18	58
F	592	22	16	9
G	620	20	20	51
G	620	22	18	49

[a]MW, molecular weight.

TABLE 3
Number of Carbon Atoms in the Ester Chain (R) and the Lactone Chain and Relative Amounts of Isomers in One Dimer from Fatty Acids with Odd Numbered Carbon Atoms[a]

		Number of carbon atoms		
Dimer	MW	Ester chain	Lactone chain	Relative amount (%)
B 2	494	13	18	8
B 2	494	14	17	5
B 2	494	15	16	13
B 2	494	16	15	20
B 2	494	17	14	22
B 2	494	18	13	23
B 2	494	20	11	9

[a]MW, molecular weight.

tion of the availability of dihydroxy acids (after incomplete hydrogenation of double bonds) combined with the availability of ester acids. In main peaks D and E, C_{18} acid is more frequently found in the lactone chain than C_{20} acid. This is assumed to be caused not by limited availability of C_{20} acid but of the smaller amounts of C_{14} and C_{16} acids. If this interpretation is correct, more of the higher dimers with C_{20} acid in the lactone part, such as a dimer C_{22}–C_{20} with MW 648, can be expected to be found in the distillation residue. This dimer could not be seen by GC (Fig. 6), while SFC (Fig. 1) demonstrated the presence of peaks with higher MW. MS of the whole dimer fraction showed that the C_{22-20} dimer (MW 648) was present. The SFC–MS data of the small late-eluting peaks provided no more information, except that the C_{24} acid (MW 368) became a dominating fragment. Thus, high MW dimers with C_{20} acid in the lactone part are expected to be found in other distillation fractions in addition to the present ones.

No C_{22} acid was ever found in the lactone part, while a C_{20} acid was found in dimers D–G. Because the 20:5ω3 fatty acid (with a double bond at C_5) is common in fish oil, together with 22:6ω3 (with a double bond at C_4), it seems reasonable to conclude that the lactones are δ-lactones. This is the expected result from a hydroxyl group at carbon 5. A hydroxyl group at carbon 4 or 5 is required for lactonization to take place; a hydroxyl group at carbon 4 will result in a five-membered lactone ring (γ-lactone), and a hydroxyl group at carbon 5 will result in a six-membered lactone ring (δ-lactone). Other ring sizes are not expected to be stable enough to survive the conditions of production. GC and SFC of such compounds on the current columns could be expected to give a retention order according to the number of carbon atoms only, hence the coelution of isomers.

The suggested formation of dimers from incompletely hydrogenated polyunsaturated fatty acids is only a hypothesis, which needs to be proved by further studies of the content of other product fractions. Because colored products can be formed in the production of fatty acids from other sources, such as tallow, it would also be interesting to establish whether dimers are present in these products too.

The colored fraction, which was not extracted by SFE, contained trimeric products with free acid groups (from MS data). The trimers are assumed to have been formed by diester formation with unsaturated hydroxy acids that are not capable of forming lactones. Due to smaller amounts available, the structures of the trimers have not been determined yet.

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