Isolation and Characterization of Polymers in Heated Olestra and an Olestra/Triglyceride Blend

Dale R. Gardner* and **Robert A. Sanders**
The Procter and Gamble Co., Winton Hill Technical Center, 6071 Center Hill Road, Cincinnati, Ohio 45224

High molecular weight components in thermally oxidized olestra (formerly called sucrose polyesters) and a mixture of olestra and soybean oil were characterized. The high molecular weight components of these oils were separated by preparative size exclusion chromatography and analyzed intact by mass spectrometry, infrared, and nuclear magnetic resonance spectroscopy. The materials isolated from the heated olestra were identified as olestra polymers. Materials isolated from the heated mixed oil (olestra and soybean oil) were identified as polymers of olestra and copolymers of olestra and triglycerides. Polymer linkages identified were identical to those resulting from thermal oxidation of natural vegetable oils of similar fatty acid composition.

KEY WORDS: Dimer methyl esters, olestra, olestra polymers, polymers, sucrose polyester, triglyceride polymers.

Some sucrose polyesters have chemical and physical properties similar to triglycerides (1). Typically, sucrose polyesters are prepared from the reaction of sucrose with long chain fatty acid methyl esters (mostly C16, C18, C18:1, C18:2, and C18:3 fatty acids). Depending upon the reaction conditions, anywhere from one (monoester) to eight (octaester) fatty acids can be attached to the sucrose molecule. Olestra is the proposed common or usual name for the mixture of hexa-, hepta- and octaesters of sucrose formed with long chain fatty acids. The specific family of olestra examined in this work consisted of predominantly octaesters (Fig. 1), but with significant amounts of the hepta- and some hexaesters.

Unlike triglycerides, olestra is not hydrolyzed by the enzymes in the gastrointestinal tract (2) and is not absorbed (3). Functionally, olestra could be used for many of the same food preparation applications as triglyceride base oils, possibly as a total replacement or in a mixture with triglycerides. Under thermal oxidative conditions, olestra should undergo the same type of polymerization reactions occurring in triglycerides of similar fatty acid composition. Polymerization would be expected to occur in heated olestra and also in heated mixtures of olestra and triglycerides.

Characterization of polymer products formed in the thermal oxidative treatment of fats and oils has been of great interest over the last thirty years. Most recently, Christopoulou and Perkins synthesized several dimer standards for characterization by chromatography and mass spectrometry (4-6). They later used these techniques to thoroughly characterize the dimers formed in heated soybean oil (7,8). Additional information can be found in reviews by Paquette (9), Nawar (10), and Figge (11). The importance of such components arises from their potential effects on oil quality during use in food preparation. It is generally agreed that, under thermal oxidative conditions, polymer formation occurs via free radical reactions between the long chain fatty esters of triglycerides. The polymers formed are large molecules, mostly dimers and trimers of triglycerides, having low volatility, and thus remain in the oil even at high temperatures. After extended use, the polymer concentration may reach levels of 5-15% of the sample. The focus of this work was to isolate and characterize any polymers formed in olestra and olestra/triglyceride mixtures heated under conditions simulating normal food preparation and the use of natural vegetable oils.

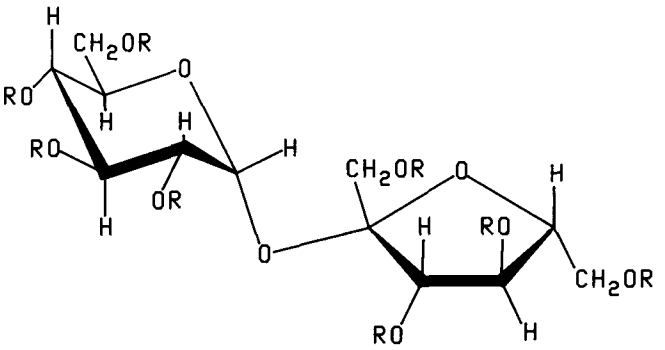

FIG. 1. Structure of octaester component of olestra. R = attached long chain fatty acid (see Table 1).

EXPERIMENTAL PROCEDURES

Materials. Batches of olestra were synthesized from sucrose and fatty acid methyl esters of partially hydrogenated soybean oil as described by Volpenhein (12). Two samples of olestra were prepared and used in this investigation. Sample A was composed entirely of olestra. The fatty acid composition is given in Table 1 and was determined by gas chromatography (13). A second olestra sample of similar composition was mixed with

TABLE 1

Fatty Acid Composition of Heated and Unheated Olestra and Olestra/Soybean Mixed Oils

Fatty acid component	Sample (A) Olestra		Sample (B) Olestra/soybean oil	
	Unheated	Heated	Unheated	Heated
%C16	11.8	11.8	9.9	10.7
%C17	—	0.2	0.2	0.2
%C18	48.3	53.2	40.1	39.9
%C18:1	21.1	20.9	25.5	26.3
%C18:2	16.6	12.6	21.6	20.3
%C18:3	1.2	0.6	1.7	1.4
%C20	0.4	0.5	0.5	0.4
%C20:1	—	—	0.2	0.0
%C22	—	0.2	0.3	0.3

*To whom correspondence should be addressed.

partially hydrogenated soybean oil (source of triglycerides) at a ratio of 75% olestra to 25% soybean oil to make sample B. The fatty acid compositions of this mixture are also given in Table 1.

Sample A was subjected to thermal oxidative conditions by heating it to 190°C in a 15-pound capacity fryer (Toastmaster 15) open to ambient conditions for six 12-hr days. Sample B, the mixture of olestra and triglycerides, was heated to 185°C for seven 12-hr days using similar 15-pound capacity fryers. Raw, french style, cut potatoes were fried in sample B throughout the seven days of heating (49 batches/day at 380 grams/batch). The fatty acid composition of both samples A and B after heating is presented in Table 1.

Size exclusion chromatography. High performance size exclusion chromatography (HPSEC) was accomplished with: A Waters 590 pump (Water Associates, Bedford, MA), Valco EC6W injector with a 20 μL injection loop (Valco, Houston, TX), 5 μ, 500 Å, 60 cm (PLGel) column, (Polymer Laboratories, Amherst, MA), and an Erma (Erma Optical Works, Ltd., Tokyo, Japan) ERC 7510 temperature controlled (40°C) refractive index detector. Tetrahydrofuran was used as the mobile phase at a flow rate of 1.0 mL/min. In order to estimate the molecular weight of components eluting from the column, the system was calibrated by injecting polystyrene standards obtained from Fisher Scientific (Pittsburgh, PA) with molecular weight values of 800, 2000, 4000, 9000, 17,500, and 50,000. Retention times were determined for each of the standards and plotted vs the log of the molecular weights.

Isolation of olestra dimers from sample A. The heated olestra (sample A) was dissolved in methylene chloride (250 mg/mL). Ten 1.0 mL aliquots of this solution were injected into a preparative SEC system consisting of a Waters 590 solvent pump, a Rheodyne injector, and a Waters stainless steel preparative column, 2.54 cm (o.d.) × 125 cm, packed with 5μ, 500 Å Styragel. Methylene chloride was used as the eluting solvent at a flow rate of 5.0 mL/min. Sample collections were made after passing the solvent flow through a refractive index detector. Three fractions were cut from the effluent, labeled A-1 to A-3, and identical fractions from successive injections were combined. The composition of each fraction, as determined by subsequent analyses, was as follows: Fraction A-1 (olestra polymers); Fraction A-2 (olestra polymers, olestra dimers, olestra monomer); Fraction A-3 (olestra monomer).

Fraction A-2 was reinjected onto the preparative SEC column and the effluent cut into ten fractions. Each fraction was sampled using the HPSEC system. Fractions 6, 7, and 8 were found to contain the highest concentration of olestra dimer and were pooled. The olestra dimer composite sample had the following actual composition—79% dimer, 18% trimer, and 3% monomer.

Preparative thin layer chromatography (PTLC) of methyl esters. The isolated olestra dimers from sample A were converted to the methyl esters by transesterification with sodium methoxide (14). The resulting methyl esters were separated by PTLC on a silica gel pre-coated glass plate (20 cm × 20 cm, 0.5 mm thick). The plate was developed twice with a mixed solvent system (90:10:2, hexane/Et_2O/HOAc). After drying, a small strip of the plate was sprayed with a 25% H_2SO_4 solution and charred on a hot plate for visualization. Four fractions were then scraped from the remainder of the plate and eluted with a MeOH/$CHCl_3$ (50:50) mixture: Fraction 1, Rf = 0.82 (methyl esters); Fraction 2, Rf = 0.63 (dimer methyl esters), Fraction 3, Rf = 0.55-0.3 (polar dimer methyl esters); Fraction 4, Rf-0.3-0.0 (very polar dimer methyl esters).

Isolation of olestra/triglyceride polymer from sample B. The heated olestra/triglyceride mixture (sample B) was dissolved in methylene chloride (30 mg/mL). Ten 150-μL aliquots were injected into a semipreparative SEC system consisting of a Waters 590 solvent pump, three PLGel columns (5μ, 500 Å, 60 cm) connected in series, and a refractive index detector. Methylene chloride was used as the eluting solvent at a flow rate of 1.0 mL/min. Six fractions were collected from each injection and identical fractions from successive injections were combined. The composition of the final six fractions were as follows: Fraction B-1, higher polymers and olestra dimers (66/44); Fraction B-2, olestra dimer and olestra monomer (52/48); Fraction B-3 (olestra-triglyceride dimer, olestra monomer, and olestra dimer (48/39/16); Fractions B-4 and B-5, olestra monomer; and Fraction B-6, triglyceride monomer. Fractions containing suspected olestra dimer and olestra-triglyceride dimer (B-2 and B-3) were then methylated by standard procedures (14).

Gas chromatography. Gas chromatography of the methyl esters was accomplished on a HP-5880 gas chromatograph equipped with capillary split injector (300°C) and FID detector (325°C). The column was a DB-5 (J&W Scientific, Folsom, CA), 30 m × 0.32 mm with a 0.10 μm film thickness. Carrier gas was high grade Helium with a column head pressure of 12 psi. The oven was temperature programmed starting at 200°C for 2.00 min, then increased to 325°C at 10 deg/min and finally held at 325°C for 10.00 min.

Dimer fatty acids (Empol 1010, C_{36} di-carboxylic acids) were obtained from the Emery Chemical Co. (Henkel-Emery Group, Cincinnati, OH). These acids were methylated with BF_3, in methanol, by standard procedures (15). The methyl esters were then chromatographed, as described above, to provide the retention time characteristics of those compounds. This information was then used to tentatively identify potential dimer methyl esters in samples derived from heated olestra and olestra/triglyceride oils.

Nuclear magnetic resonance and infrared analyses. Nuclear magnetic resonance (NMR) and infrared (IR) spectra were obtained on the preparatively isolated olestra monomer and dimer samples from sample A. NMR spectra were obtained on a General Electric (GE NMR Instruments, Fremont, CA) QE-300 Fourier transform spectrometer at a proton frequency of 300 MHz and carbon-13 frequency of 75.5 MHz. Samples were dissolved in deuterated chloroform and referenced to signals arising from residual nondeuterated solvent (7.26 ppm for proton and 77.0 ppm for carbon). Diffuse reflectance IR spectra were obtained on a Digilab FTS-15E Fourier transform infrared spectrometer (Bio-Rad, Cambridge, MA). Samples were deposited on powdered KBr and analyzed at a resolution of 4 cm^{-1}.

Mass spectrometry. Plasma desorption mass spectrometry (PDMS) of intact olestra polymers was performed using a Bio Ion 20 PDMS system (Applied Bio

Systems AB, Uppsala, Sweden), as previously described (16). Mass spectrometry of methylated dimer fatty acids was performed on a Kratos MS-30 system (Kratos Anayltical, Ramsey, NJ) operated in the double beam mode at low mass resolution and 3 second/decade scan rate. Electron ionization (EI) spectra were taken at 70 eV. Isobutane was used as the reagent gas for chemical ionization. Sample introduction was via a heated solids probe. Gas chromatography/mass spectrometry (GC/MS) was performed on a Hewlett Packard HP-5995A (Hewlett-Packard, Palo Alto, CA) system in the EI mode at 70 eV. Samples were injected in the splitless mode and all other GC conditions were identical to those stated above.

RESULTS AND DISCUSSION

Size exclusion chromatography of heated olestra. The size exclusion chromatogram of the unheated olestra (sample A) is displayed in Figure 2A. Figure 2B is the size exclusion chromatogram of the olestra after heating for six days. The heated material clearly shows an increase in high molecular weight components as evidenced by peaks at retention times of 11.47 and 12.20 min. To obtain approximate molecular weights (MW), retention times were compared to polystyrene standards. Peak 1 (RT = 13.15) corresponds to olestra monomer material and the MW was estimated to be 2,780. The MW of the other peaks were approximated to be 6,033 for peak 2 (RT = 12.20) and 8,728 for peak 3 (RT = 11.47). These weights correspond well with values of increasing multiples of the original olestra monomer material. For example, peaks 2 and 3 correspond to olestra dimer and trimer, respectively. Accurate molecular weights for the olestra polymer components were better obtained by plasma desorption mass spectrometry.

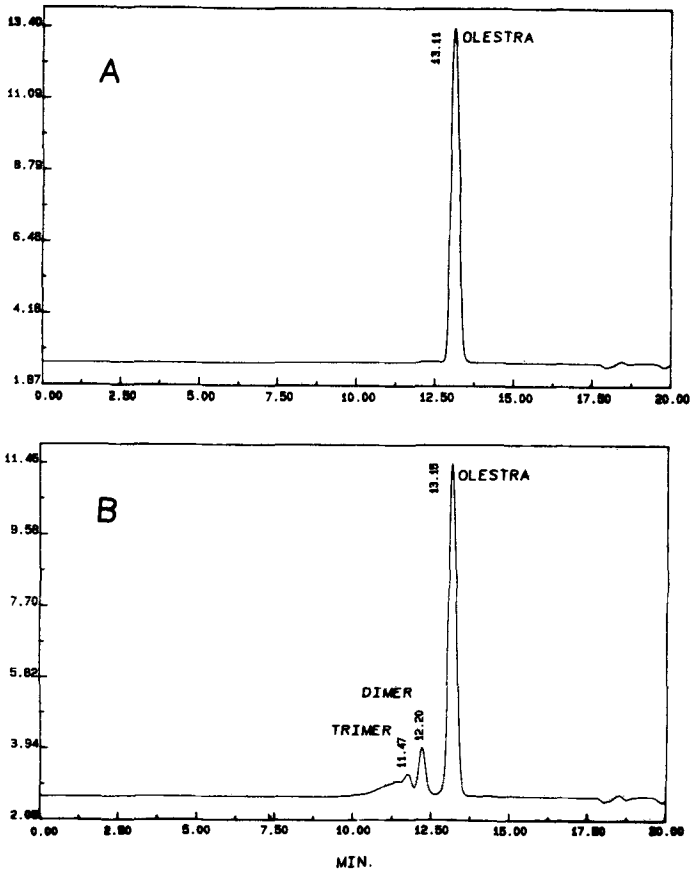

FIG. 2. A, HPSEC chromatogram of unheated olestra sample A; B, HPSEC chromatogram of heated olestra.

PDMS characterization of isolated olestra dimer from sample A. Olestra monomers (RT = 13.15 min) and the olestra dimers (RT = 12.20) were isolated using preparative size exclusion chromatography. Mass spectra of these components were then acquired using plasma desorption mass spectrometry. A complete interpretation of the data is beyond the scope of this publication, but can be found, in part, along with copies of the spectra, in the proceedings of the 36th ASMS Conference (16). A full report on desorption mass spectrometry of sucrose esters is in preparation. For the isolated dimer material, a mass of 4898 daltons was observed. This was interpreted as a Na-adduct ion for an octaester dimer with a mixture of C16 and C18 fatty acids (NaI is added to the sample matrix to enhance molecular ion stability through sodium adduction). Corresponding molecular ions were observed for the isolated monomer material at approximately 2464 daltons. These results confirm that material eluting at 12.20 min and 13.15 min from the size exclusion chromatograph (Fig. 2B) are olestra dimer and monomer, respectively.

IR and NMR characterization of olestra dimer from sample A. IR, carbon-13 and proton NMR data were acquired on the isolated olestra dimer mixture. For comparison, reference spectra of the olestra monomer (fraction A-3) were also obtained. In all cases, spectra of the olestra dimer material were virtually identical to those of the monomer with respect to the number of resonances and the wavelength, or chemical shift, at which they occurred. It can therefore be concluded that the isolated high molecular weight material is olestra-like. That is, the basic chemical composition consists of sucrose esterified with long chain fatty acids, and that the larger molecular size must result from the combining of two or more olestra molecules in a fashion not altering the basic sucrose backbone structure.

The IR and proton NMR spectra are displayed in Figures 3A and 3B (no new information was evident from the carbon-13 NMR data, and therefore this data is not displayed). The only real difference between spectra of the monomer and dimer was broadening of the NMR resonances belonging to the sucrose moiety of the isolated olestra dimer (see the expanded portions of the proton NMR spectra in Fig. 3C). This line broadening could be caused by shorter T_2 relaxation. Shorter T_2 relaxation could result from decreased molecular motion (increase in viscosity) that accompanies polymerization.

The combined data of molecular weight, as determined by PDMS, IR, and NMR, supports the conclusion that the high molecular weight compounds are indeed olestra dimers. The type of polymer linkage between the two olestra molecules is not directly evident from these data. What does appear to be evident is that the sucrose backbone structure is not changed upon polymerization. If one assumes that the chemistry of heated olestra materials is similar to that of heated triglycerides, then one would expect dimer linkages to form between intermolecular fatty ester side chains. Such a linkage, far

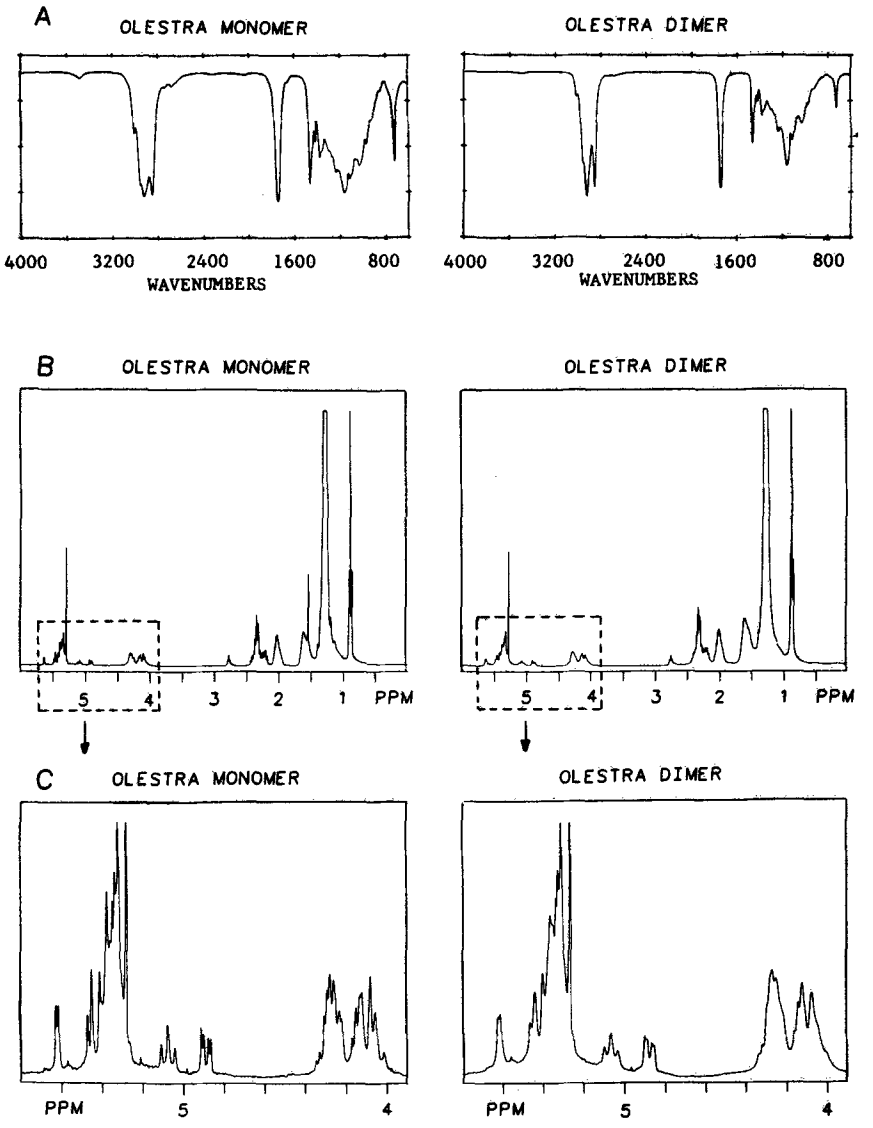

FIG. 3. Infrared and proton NMR spectra of isolated olestra monomer and dimer. A, Infrared spectra; B, Proton NMR spectra; and C, expanded plot of proton NMR spectra displaying sucrose backbone resonances.

removed from the sucrose molecule, would result in little or no change in the sucrose NMR resonances.

Gas chromatography of methylated dimer acids. The general approach to identifying the polymer linkages in triglycerides has been to transesterify the heated oils and characterize the resulting methylated dimer fatty acids by GC and MS techniques. A similar approach was applied to the heated olestra sample.

Portions of the isolated olestra dimer and monomer materials were transesterified with sodium methoxide. The methyl esters were extracted with hexane and then analyzed by GC. For those methyl esters from the transestered olestra dimers, a number of compounds eluted at retention times similar to those of the methylated C_{36} dicarboxylic acids (Empol 1010 dimer fatty acids from the Henkel-Emery Group). Methyl esters from the olestra monomer material also contained components with retention times appropriate for dimer methyl esters, although their quantities were much less than these observed from the olestra dimer methyl esters. The presence of dimer methyl esters in this fraction probably results from intramolecular dimerization.

It was clear that in order to identify specific dimer methyl esters that further separation of the dimer methyl esters from the mono methyl esters would be necessary. Therefore, methyl esters resulting from the transesterification of isolated olestra dimer were subjected to preparative TLC procedures as outlined in the experimental section. Fractions were labeled as either apolar or polar dimers similar to the scheme used by Ottaviani *et al.* (17). Each fraction was analyzed by gas chromatography and the resulting chromatograms are displayed in Figure 4. As shown in this Figure, fraction #2, the apolar dimers, contained the majority of the methylated dimer fatty acids.

Identification of apolar dimer linkages. Mass spectra of the apolar dimer methyl esters (PTLC fraction #2) obtained from the heated solids probe are shown in Figure 5. Both CI, where $(M+H)^+ = 587$ is observed, and EI, where $M^+ = 586$ is observed, indicate that the major

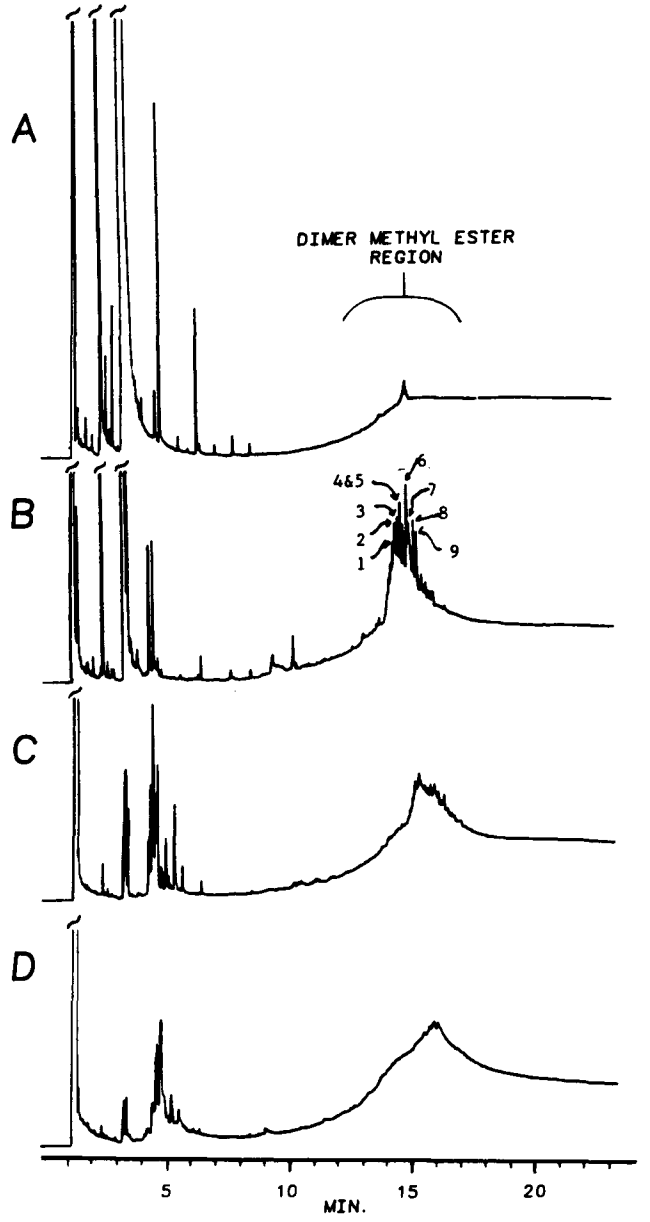

FIG. 4. GC chromatograms of preparative TLC fractions from separation of methyl esters of olestra dimers. A, Fraction #1, monomethyl esters; B, Fraction #2, apolar dimer methyl esters; C, Fraction #3, polar dimer methyl esters; and D, Fraction #4, very polar dimer methyl esters.

component has a molecular weight of 586. This molecular weight is correct for a methylated dicarboxylic dimer fatty acid ($C_{38}H_{66}O_4$). A large fragment ion was present at 293 (M/2), indicating symmetry resulting from a simple carbon-carbon dimer linkage in the molecule. Other significant fragment ions found were 555, 515, 489, 475, 429, 403, 389, and 319. The data matched that reported for the dehydro dimers of methyl linoleate (8,17,18). The dehydro dimer structure is displayed in Figure 6I.

Data from GC/MS analysis of the apolar dimers (PTLC fraction #2) are presented in Table 2. The Table lists the major ion fragments observed above *m/z* 250 in spectra taken from nine partially resolved peaks displayed in Figure 4B. The relative intensities of the fragments are listed in parentheses (normalized to the most abundant ion above *m/z* 250). Peaks 1-5 were identified as isomers of the dehydro dimer of methyl linoleate (Fig. 6I). Their mass spectra were virtually identical to each other and to that reported in the literature (8,17,18) for this compound. Data for peak 6 were almost identical to that reported by Christopoulou (8) for a bicyclic dimer of methyl linoleate (Fig. 6II). Peaks 7-9 yielded mass spectra suggestive of additional cyclized species. Cyclization destroys the symmetry present in the dehydro type structure, (Fig. 6I) and fragmentation yielding the dominant *m/z* = 293 fragment (M/2) of the single carbon-carbon linkage is no longer possible. A number of cyclic isomers are possible. For a C_{36} dimer methyl ester of mw = 586, the number of double bonds and/or rings must total four (19). Therefore, a number of different isomers would be possible including monocyclic, other bicyclic, and even tricyclic structures. The exact nature of the dimers represented by peaks 7-9 is unknown at this time and would be impossible to determine solely on the GC/MS data obtained here without reference to spectra of known compounds.

From the mass data above, it appears that linoleic acid (C18:2) is the key component of these dimer linkages. Polymerization of the esterified linoleic acid is supported by changes in the fatty acid composition (FAC) during thermal oxidation. In Table 1, the fatty acid composition of the olestra material is given before and after the six days of heating at 190°C. Of interest is the significant drop in the linoleic acid composition (16.6% fresh vs 12.6% heated). This drop can be attributed to the formation of C18:2 fatty ester dimer linkages in the olestra material. It can also be observed that the two major fatty acids present in the sample, stearic and oleic acid, do not show any appreciable reduction over the seven days. These data are consistent with the observation that neither of these two fatty acids is found as a major component of the dimer linkages.

Identification of polar dimer linkages (PTLC fraction #3 and #4). CI mass spectra collected from PTLC fraction #3, the polar dimers, indicated that the major species had molecular weights of 602 and 604. Such molecular weights can be interpreted as forms of oxygenated dimer species evidenced by an increase of 16 and 18 mass units, respectively, relative to the apolar dimers. Important fragment ions at *m/z* 293, 309, and 311 help support this hypothesis. These ions result from cleavage of the single dimer linkage between individual fatty ester units. The 293 ion can be attributed to a methyl linoleate type fragment, similar to that seen in the mass spectra of the dehydro dimer of methyl linoleate. Fragments at 309, 311 arise when the charge is retained on the other portion of the molecule. The presence of oxygen in these fragments is indicated again by the increase in 16 (*m/z* 309) and 18 (*m/z* 311) mass units. Such dimer linkages have been previously reported and include an ether bridge dimer (Fig. 6III) described by Ottaviani (mw = 602), a ketone containing dimer (Fig. 6IV; mw = 602), and a hydroxylated dimer (Fig. 6V; mw = 604 identified by Christopoulou and Dobarganes *et al.* (8,18). The mass data alone are not capable of readily distinguishing among these different possibilities. Attempts to further characterize this fraction by other spectroscopic techniques were limited because of the small quantities of materials isolated.

The problem of characterization of the dimer linkage

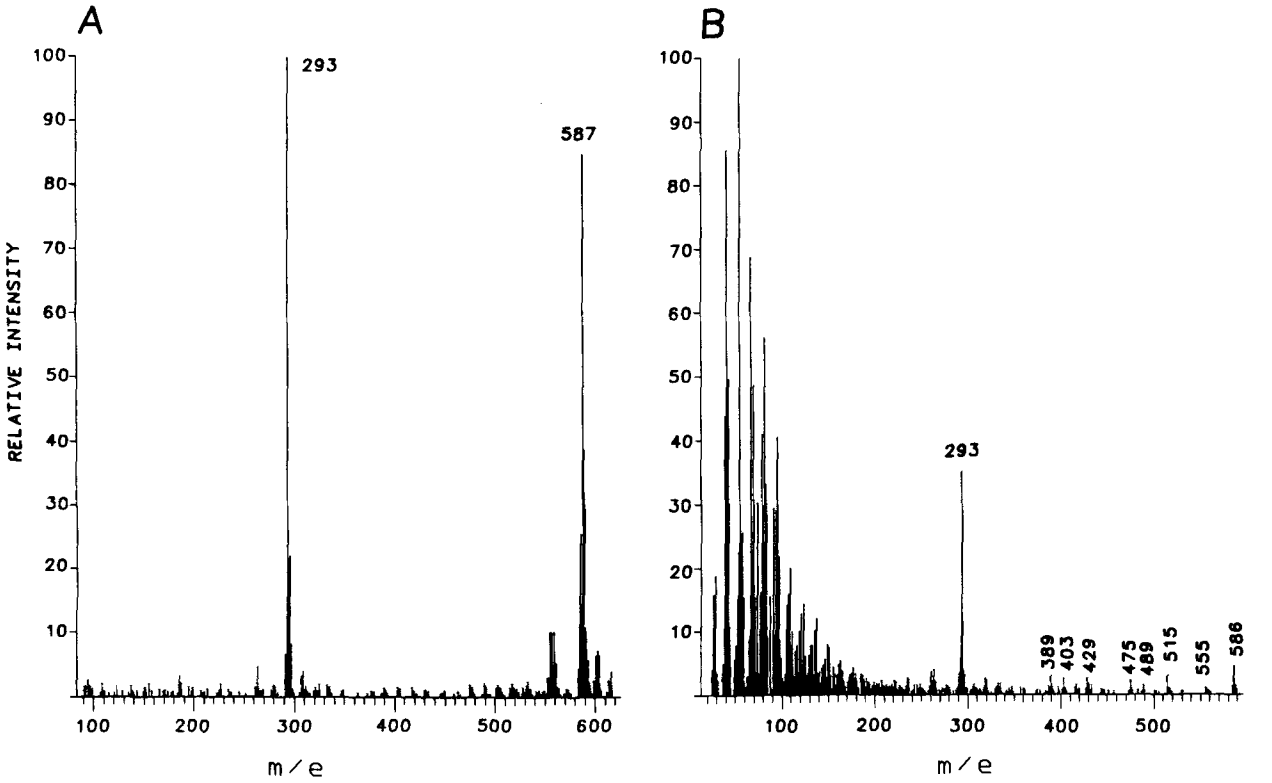

FIG. 5. Mass spectra of apolar dimer methyl esters (PTLC fraction #2). A, Isobutane CI spectrum; and B, EI mass spectrum (70 eV).

was even more pronounced in PTLC fraction #4. Only CI mass data were informative and indicated that the isolated components were of even greater molecular weight than those in fraction #3 (ionization was observed at *m/z* 615-621, 631, 632). One possible explanation for these masses is that the components are multi-oxygenated dimer species. For example, Christopoulou and Perkins reported finding the dihydroxy dehydro dimer of methyl linoleate (mw = 620) in their work on heated soybean oil (7).

Characterization of methyl esters produced from isolated olestra dimers has shown that the major dimer linkage occurs as a simple carbon-carbon bond between fatty ester chains of the olestra molecules. The resulting olestra dimer structure, containing such a linkage, is shown in Figure 7. There are a number of different isomers which may occur depending on the location of this carbon-carbon dimer linkage. Only one is shown here.

Characterization of olestra/triglyceride polymer. This work was performed in order to establish the nature of polymers which form upon heating olestra/triglyceride (TG) mixtures. In mixed olestra/triglyceride systems, one would predict the formation of mixed dimers (olestra-triglyceride) in addition to the olestra and triglyceride dimers observed in pure systems. A size exclusion chromatogram of the heated olestra/triglyceride mixed oil is shown in Figure 8B. Again, the formation of polymers is evident from the earlier eluting peaks (retention times of 12.18 and 12.62), which are absent in the unheated mixture. Comparison of heated olestra/triglyceride oil to heated olestra (Fig. 2B) reveals a polymer peak in the heated mixed oil not present in the olestra pure oil. This peak (RT = 12.62 min) is found between the olestra monomer and olestra dimer peaks at a retention time consistent with the expected olestra-triglyceride dimer. These polymer peaks were isolated using the semipreparative SEC system described in the experimental section. Triglyceride dimers are also expected to form upon heating the olestra/triglyceride mixed oil. However, it was not possible to isolate this component because the molecular weight of the triglyceride dimer is similar enough to olestra monomer that the two components were not resolved.

PDMS analysis of olestra-triglyceride polymers isolated from sample B. The isolated polymer components, fractions B-2 and B-3, were analyzed by plasma desorption mass spectrometry. The mass spectrum of fraction B-2 (RT = 12.18 min on HPSEC, Fig. 8B) identified that peak as olestra dimer material as predicted. It was similar to that isolated from the heated non-triglyceride containing olestra. For fraction B-3 (RT = 12.62), a mass of 3358 Da was observed identifying this material as olestra-triglyceride dimer. The expected mass of a dimer formed from the octaester component of olestra and a triglyceride adducted with sodium cation is 3343.

MS characterization of dimer methyl esters. Methylation of fractions B-2 (olestra dimer) and B-3 (olestra-triglyceride dimer) produces dimer fatty acid methyl esters from those fatty acids actually involved in the olestra and olestra-triglyceride dimer linkages. Previously, we separated the mono methyl esters from the dimer methyl ester by preparative TLC, but because of the small amount of material isolated from the heated olestra/triglyceride oil, a different approach was chosen. The procedure was similar to that described by Jensen and Moller (20). Distillation of the methylated fractions (B-2

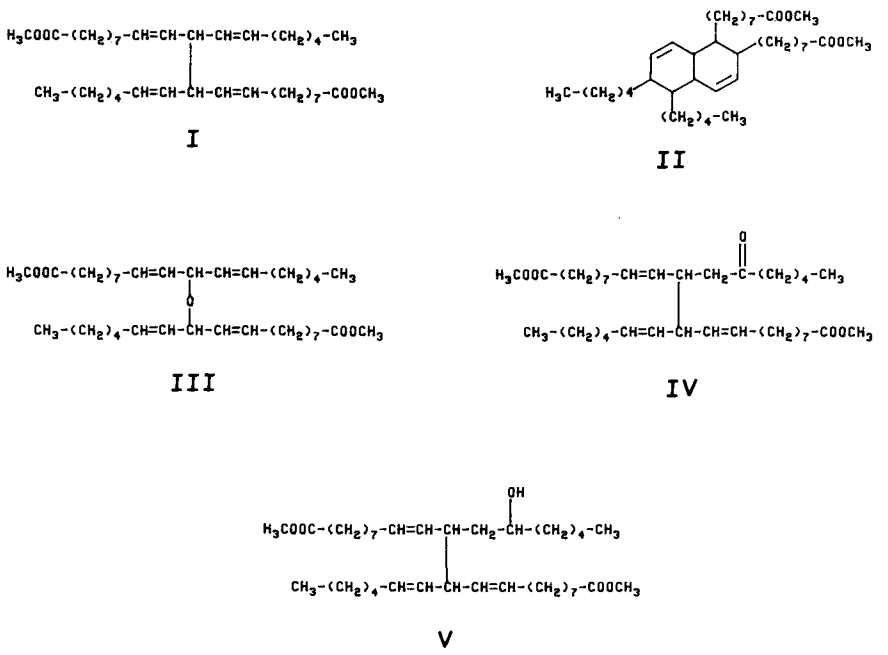

FIG. 6. Dimer methyl esters. I, Dehydro dimer of methyl linoleate; II, bicyclic dimer of methyl linoleate; III, ether bridged dimer; IV, keto dimer; and V, monohydroxy dimer.

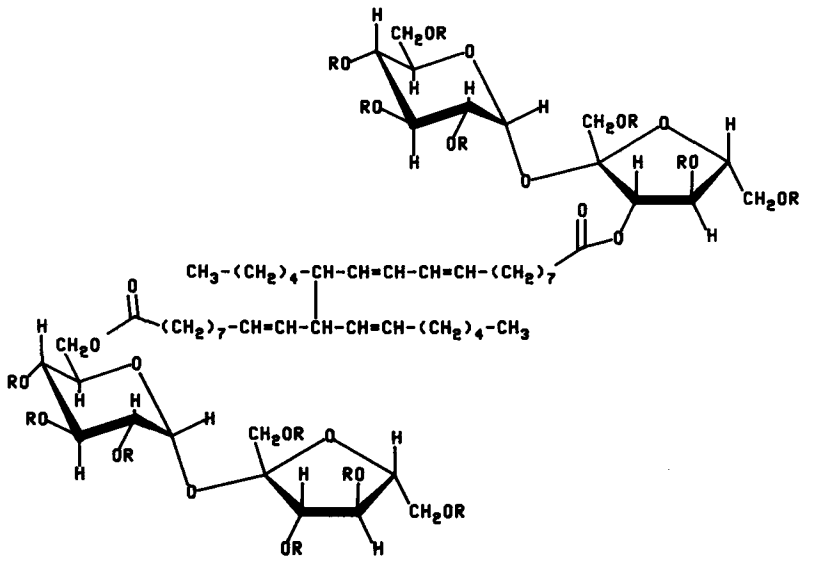

FIG. 7. Structure of major olestra dimer formed from two octaester species. R = attached long chain fatty acid (see Table 1).

and B-3) from the MS solids probe produced a large ion current at relatively low temperature corresponding to the monomeric esters and smaller currents at higher temperatures corresponding to the dimeric esters. The CI and EI mass spectra of the higher molecular weight methyl esters from fraction B-3 (olestra-triglyceride dimer) are very similar to Figures 5A and 5B (a mixture of apolar dimers isolated from heated pure olestra), and are consistent with the structure of dehydro dimers derived from methyl linoleate (Fig. 6I). The major molecular ion is 586 (587 for isobutane CI), and the large 293 fragment ion is also present. The major difference from Figure 5 is the presence of polar dimers (molecular weight 602) in fraction B-3 that were largely removed by TLC of the methyl esters from pure olestra dimers. The methylated fraction B-2 (olestra dimer from heated olestra/triglyceride oil) CI and EI spectra were indistinguishable from those of methylated fraction B-3 (olestra-triglyceride dimer). These data indicate that the linkages are identical between olestra dimers and olestra-triglyceride dimers. A proposed structure of the major olestra-triglyceride dimer would then be as shown in Figure 9. Again, only one of several isomers is shown.

That the higher molecular weight species in heated

olestra are polymers is strongly supported by direct molecular weight measurement (PDMS and size-exclusion chromatography). These polymers are linked through the fatty ester moieties as revealed by methylation of the polymeric fractions followed by GC/MS. The dominant fatty acid linkage in olestra dimer is the same as that observed in triglyceride polymers, a carbon-carbon bridge to form the dehydro dimer. Other linkages are also present, including multicyclic linkages and dimer species with oxygen-containing functional groups. Mixed polymers form in mixtures of olestra and triglycerides, as determined by SEC and PDMS as well as by MS analysis of methylated fractions. All of the polymer linkages were found to be similar to those reported in the literature for triglycerides of similar fatty acid composition. Of all the fatty acid groups present in the sample studied, linoleic acid (C18:2) appeared to be the major contributor in the polymerization process.

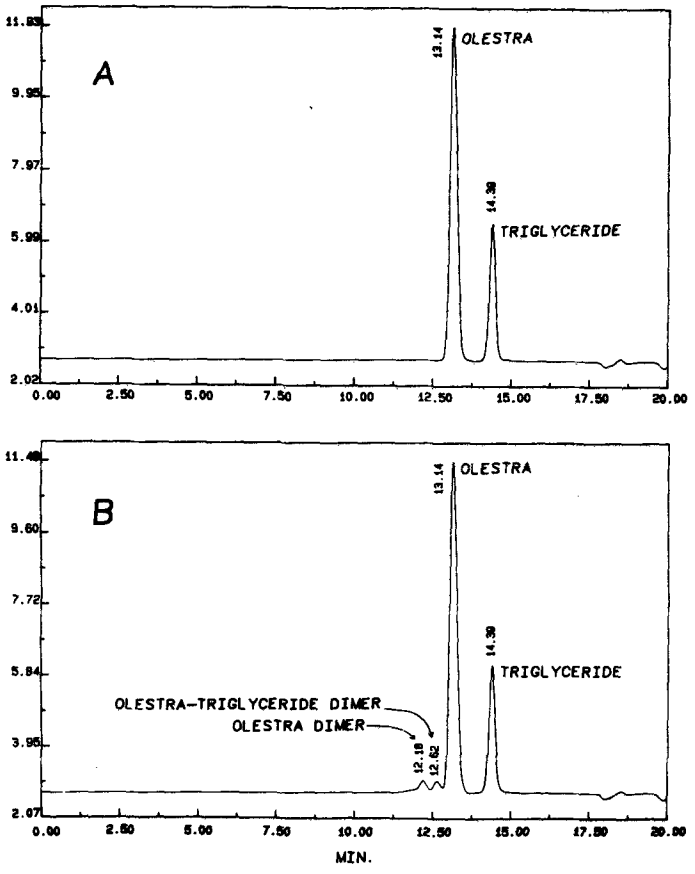

FIG. 8. A, HPSEC chromatogram of unheated olestra/triglyceride mixture; and B, HPSEC chromatogram of heated olestra/triglyceride mixture.

ACKNOWLEDGMENTS

Plasma desorption mass spectra were provided by T. Keough and M. Lacey; GC/MS and MS analyses were performed by J. Baumgardner and T. Morsch; molecular weight calibration of the HPSEC system was accomplished by S. Daniels; and IR analyses were provided by C. Damo.

REFERENCES

1. Jandacek, R., and R. Webb, *Chem. Phys. Lipids 22*:163 (1978).
2. Mattson, F., and R. Volpenhein, *J. Lipid Res. 13*:325 (1972).
3. Mattson, F., and G. Nolen, *J. Nutr. 102*:1171 (1972).
4. Christopoulou, C.N., and E.G. Perkins, *J. Am. Oil Chem. Soc. 66*:1338 (1989).
5. Christopoulou, C.N., and E.G. Perkins, *Ibid. 66*:1344 (1989).
6. Christopoulou, C.N., and E.G. Perkins, *Ibid. 66*:1353 (1989).
7. Christopoulou, C.N., and E.G. Perkins, *Ibid. 66*:1360 (1989).
8. Christopoulou, C.N., *Chemical and Chromatographic Studies on Dimeric Fatty Acids*, Dissertation, University of Illinois, 1986.
9. Paquette, G., D.B. Kupranycz and F.R. van de Voort, *Can. Inst. Food Sci. Technol. J. 18*:197 (1985).
10. Nawar, W.W., *Food Chemistry*, 2nd ed., Dekker, New York, pp. 139-244, 1985.
11. Figge, K., *Chem. Phys. Lipids 6*:164 (1971).
12. Volpenhein, R., U.S. Patent 4,517,360 (1985).
13. Slover, H.T., and E. Lonza, *J. Am. Oil Chem. Soc. 56*:933 (1979).
14. Radin, N.S., A.K. Hajra and Y. Akakori, *J. Lipid Res. 1*:250 (1960).
15. Metcalfe, L.D., and A.A. Schmitz, *Anal. Chem. 33*:363 (1961).

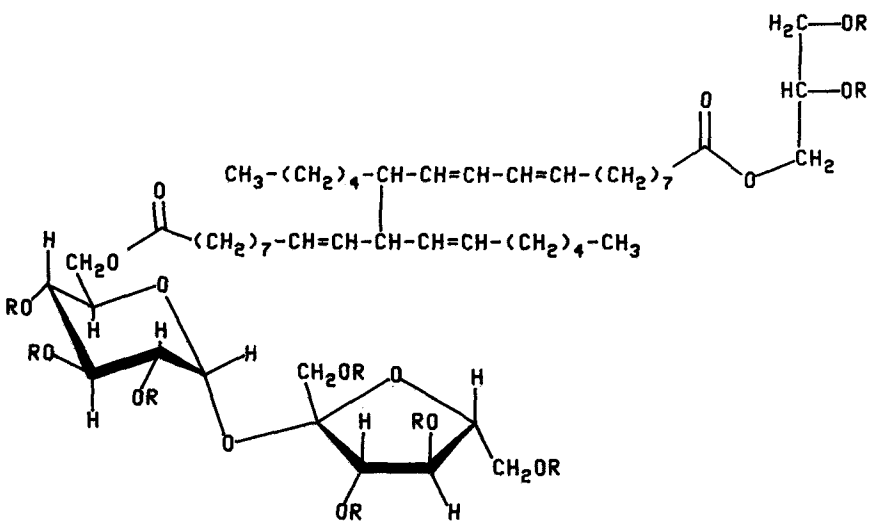

FIG. 9. Structure of major olestra-triglyceride dimer formed from octaester and triglyceride components. R = attached long chain fatty acid (see Table 1).

16. Keough, T., M. Lacey, R. Sanders and D. Gardner, *Plasma Desorption Mass Spectrometry of Sucrose Polyesters*, Proceedings of the 36th ASMS Conference on Mass Spectrometry and Allied Topics, San Francisco, CA, June 5-10, pp. 763-764, 1988.
17. Ottaviani, P., J. Graille, P. Perfetti and M. Naudet, *Chemistry and Physics of Lipids 24*:57 (1979).
18. Dobarganes, M.C., M.C. Perez-Camino and J.J. Rios, *Grasas y Aceites 35*:351 (1984).
19. Wheeler, D.H. and J. White, *J. Am. Oil Chem. Soc. 44*:298 (1967).
20. Jensen, O.N., and J. Moller, *Fette Seifen Anstrichm. 88*:352 (1986).

[Received January 16, 1990; accepted June 8, 1990]