Dimer Acids: Synthesis and Mass Spectrometry of the Tetrahydroxy, Dihydroxy, and Diketo Dimers of Methyl Stearate

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Dimers, structurally representative of those formed during thermal-oxidative reactions in fats and oils, including the dihydroxy, tetrahydroxy, and the diketo dimer of methyl stearate, were synthesized to be used as standards and model compounds for the development of various chromatographic methods needed for further studies. The mass spectra of both the methyl ester and trimethylsiloxy derivative of the synthetic dimeric compounds were investigated in order to facilitate development of methodology for the separation and structural determination of similar polar dimer structures isolated from actual oxidized or "abused" oil samples.

Evidence for the formation of polar as well as nonpolar dimers as a result of the thermal-oxidative treatment of frying fats has been previously presented (1-10). However, the determination of the individual dimeric components of thermally oxidized fats and oils has been hampered because of difficulties encountered during the isolation of dimers from the used fats as well as the complexity of the structures present in the dimeric fraction.

To aid in the determination of the dimer structures, model systems of either pure fatty acids or triglycerides oxidized under simulated deep fat frying conditions have been employed (11-13). Still, the methods for the determination of individual polar dimeric structures are not available and are usually limited only to the determation of molecular weights, oxygen content, and unsaturation. To date, gas chromatography has usually yielded one peak containing a complex mixture of dimers.

In the present study, the synthesis of dimers containing hydroxy and keto groups, representative of those which may be formed during thermal oxidative reactions in fats and oils, and their mass spectral analysis is presented. These dimers were required for subsequent studies for the development of chromatographic methodology needed for dimer isolation and for the structural determination of dimers from oxidized oil samples.

EXPERIMENTAL

Synthesis of tetrahydroxystearate dimer. The tetrahydroxystearate dimer was prepared by peroxidation of the dehydrodimer of methyl oleate which was prepared according to Paschke *et al.* (14). Peracid oxidation of the double bonds was carried out according to the method described by Swern *et al.* (15) as follows:

One ml of 30% (100 volume) hydrogen peroxide was added to a well stirred mixture of 380 mg (0.0006 mol) of the dehydrodimer of methyl oleate and 2 ml of formic acid in a 50 ml three-necked flask at 25°C. The reaction became mildly exothermic after a lag of about 5 min, and homogeneous after 15 min. The temperature was maintained at a 40°C constant. The peroxide content of the reaction mixture was checked periodically as follows: a small sample of the reaction mixture (0.05 ml) was dissolved in glacial acetic acid (A.C.S. grade, Fisher Scientific Company, Fair Lawn, NJ). The presence of peroxides was indicated by the addition of a few drops of 10% solution of sodium iodide in acetone, and the development of a yellow or brown color when the concentration of peroxides in the sample was low or high, respectively.

After about five hours when analysis has indicated that the peroxide has been consumed, the formic acid was removed by pouring the reaction mixture into 100 ml of water and extracting the oily phase three times with 50 ml volumes of a mixture of ethyl ether (E.M. Science, Cherry Hill, NJ) and hexane (glass distilled, Burdick & Jackson Laboratories, Inc., Muskegon, MI), (3:1, v/v). The organic layers were combined and washed with water until free of formic acid (pH ~7, wash). The solvent was then removed with a rotary evaporator (Flash Evaporator, Buchler instruments, Fort Lee, NJ).

The residue in the flask, which consisted of the dimeric hydroformoxy-stearic acids, was heated for 1.5 hours at 100°C with a 100% excess of aqueous sodium hydroxide (E.K. Industries, Inc., Addison, IL). The hot, amber-colored soap solution was cautiously poured into an excess of 3N hydrochloric acid and stirred. The product was extracted with a mixture of ethyl ether and hexane (3:2, v/v). After evaporation of the solvents, the product was collected and dried under vaccum. The yield was 213.7 mg which was 56% based on the amount of the dehydrodimer of methyl oleate used.

Synthesis of dihydroxystearate dimer. The dihydroxystearate dimer was synthesized by the addition of anhydrous formic acid, in the presence of strong acidic catalyst, to the dehydrodimer of methyl oleate prepared according to the method of Knight *et al.* (16).

A mixture of 117 mg (0.002 mol) of dehydrodimer of methyl oleate, 0.4 ml of anhydrous formic acid (Allied Chemical, Morristown, NJ), and 0.05 ml of 70% aqueous perchloric acid was refluxed for 15 min in an atmosphere of nitrogen. The mixture became homogeneous between 95 and 100°C. After the termination of the reaction, the excess formic acid was removed by pouring the reaction mixture into 50 ml of H_2O, as well as extraction of the oily phase three times with 30 ml of a mixture of ethyl ether-hexane (3:1, v/v). The solvent was then removed with a rotary evaporator. The residue was subsequently heated at 120°C for 15 min with a 100% excess of 6N sodium hydroxide, and then slowly poured into an excess of 6N hydrochloric acid and stirred. The product was extracted with a mixture of ethyl ether-hexane (3:2, v/v) and the solvent was removed by evaporation under vacuum. The yield was approximately 72% based on the amount of the dehydrodimer of methyl oleate used.

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[1]Mass spectra are available from the author upon request.

Synthesis of diketostearate dimer. The diketostearate dimer was prepared by oxidation of the dihydroxystearate dimer with the method described by Ross *et al.* (17).

Twenty-two mg of dihydroxystearic dimer was dissolved in 200 μl of acetic acid (A.C.S. grade, Fisher Scientific Company, Fairlawn, NJ), and a solution of 15 mg of chromic oxide (Mallinckrodt Inc., St. Louis, MO) in 200 μl of 90% acetic acid was added. The mixture was then heated to 350°C with stirring. Two hours later the mixture was poured into water and extracted with a mixture of ethyl ether-hexane (1:1, v/v). After the product was dried over anhydrous sodium sulfate (Mallincrodt Inc., St. Louis, MO) the solvent was removed and 15 mg of final product was obtained.

Derivative preparation. Methyl esters of the final product from the synthesis of tetrahydroxytearic, dihydroxystearic, and diketrostearic dimers were prepared according to the AOCS Method Ce 1-62 (18).

Trimethylsiloxy (TMS) derivatives were prepared according to the following procedure: Four hundred μl of pyridine (A.C.S. grade, Mallinckrodt, Paris, KY) and 100 μl of bis (Trimethylsily)Trifluoroacetamide (Supelco Inc., Bellefonte, PA) were added to 1–5 mg of sample in a 1 ml screw-capped glass vial fitted with teflon lined septum (Supelco, Inc.). The vial was then capped, shaken, and heated for 20 min at 60°C. The solution of TMS derivatives was used within 4 hr.

Chromatographic analysis. Samples were analyzed by gas liquid chromatography (GLC) and thin-layer chromatography (TLC). GLC was accomplished using a glass column 1 ft × 2 mm i.d. with 3% OV-1 coated on 80/160 mesh Supelcoport (Supelco, Inc.). The operating parameters were: initial temperature and time 120°C and 1 min, respectively; temperature rate 8°C/min, final temperature and time 350°C and 20 min, respectively. Injector and detector (FID) temperature was 360°C. Nitrogen flow rate was 60 ml/min. TLC was carried out on Silica Gel G plates using a solvent system of hexane-ethyl ether (6:4, v/v). Liquid chromatography (LC) was employed in order to separate the unreacted dehydrodimer of methyl oleate from the dihydroxy and tetrahydroxystearate dimers.

Product purification. Separation was performed on Seppac silica gel cartridges (Waters Associates, Taunton, MI). The final product (10–20 mg) of the synthesis of either the tetrahydroxy or dihydroxystearate dimers were introduced onto the cartridge and eluted with 5 ml hexane, 5 ml hexane-ethyl ether (1:1, v/v), and 10 ml ethyl ether. Fractions of 1 ml were collected and analyzed by TLC as described above. Fractions were combined according to TLC data to yield pure dihydroxy and tetrahydroxystearate dimers.

Infrared spectrophotometry. Infrared spectra were determined with a Beckman Model IR 4210 Infrared spectrophotometer. Samples were analyzed as thin films on NaCl plates.

Mass spectrometry. GC-MS analysis was performed with a Hewlett-Packard Model 5985B GC-MS (Hewlett Packard Co., Avondale, PA). The mass analyzer was a quadruple type and the electron impact ion source set at 70 eV. The chemical ionization gas used as methane. The GC conditions employed are given above.

Both the electron and the chemical ionization spectra of the tetrahydroxystearic and dihydroxystearic dimers as methyl esters, trimethylsilyloxy-derivatives of the methyl esters, and trimethylsilyl derivatives were obtained. Similarly, the electron and chemical ionization spectra of the methyl ester of the diketrostearic dimer were obtained.

RESULTS AND DISCUSSION

The reaction resulting in the formation of tetrahydroxystearate dimers from the dehydrodimers of methyl oleate is presented in Figure 1. It involves direct oxidation of the dehydrodimer by performic acid and subsequent ring opening of the epoxide to form hydroxyformoxystearate dimers.

Peracid oxidation is a direct *cis* addition, but inversion generally accompanies ring opening so that the overall result is *trans* addition. Paschke *et al.* (14) reported that per molecule of the methyl oleate dehydrodimer 1.24 of the double bonds are *trans*, with the remaining 0.76 double bonds being *cis*. Thus 1.24 of the double bonds of the methyl oleate dehydrodimer will be *cis* hydroxylated with *trans* hydroxylation of the 0.76 remaining double bonds. Addition of NaOH will give the salt of tetrahydroxysteric dimeric acid, which by acidolysis and esterification gave the tetrahydroxystearate dimer.

GLC analysis of the methyl ester of the final product of the synthesis of the tetrahydroxystearate dimer gave three main peaks. The first had a relative retention time similar to that of the dehydrodimer of methyl oleate (RRT = 2.23), whereas the second and third peaks had relative retention times of 4.43 and 4.82, respectively. When the TMS derivatives were prepared and analyzed a major peak at RRT = 2.55 was obtained, with another peak eluted at the retention time of the dehydrodimer of the oleic acid as TMS derivatives. TLC analysis gave R_f values of 0.39 and 0.80 for the tetrahydroxystearate and the dehydrodimer of methyl oleate, respectively. Liquid chromatographic purification resulted in pure tetrahydroxystearate dimer as determined by GLC and TLC. This dimer was subsequently used in various structure determination studies.

The infrared spectrum of this purified dimer is presented in Table 1. The infrared spectrum was identical to that of the methyl oleate except for the presence of strong peaks at 3445 and 935 cm^{-1}, which were representative of free hydroxy groups and of all hydroxyl groups in the molecule. The three different types of derivatives used in the mass spectrometric studies are shown for one of the isomers of tetrahydroxystearic dimer in Figure 2. Since each derivative is expected to have a different fragmentation pattern, information derived from each mass spectrum provided additional evidence for the structural characterization of the synthetic dimer.

The electron ionization (EI) mass spectrum of the methyl ester of the tetrahydroxystearic dimer did not show the expected parent peak at m/e = 658. Instead, a weak ion was observed at m/e $[M - (H_2O + CH_3OH)]^+$ as was expected for such hydroxy esters.

The most important ions obtained may be explained by cleavage of the hydroxyl groups and loss of either alkyl or ester groups from the molecular ions accompanied with hydrogen rearrangement as presented in Table 2. Ions at

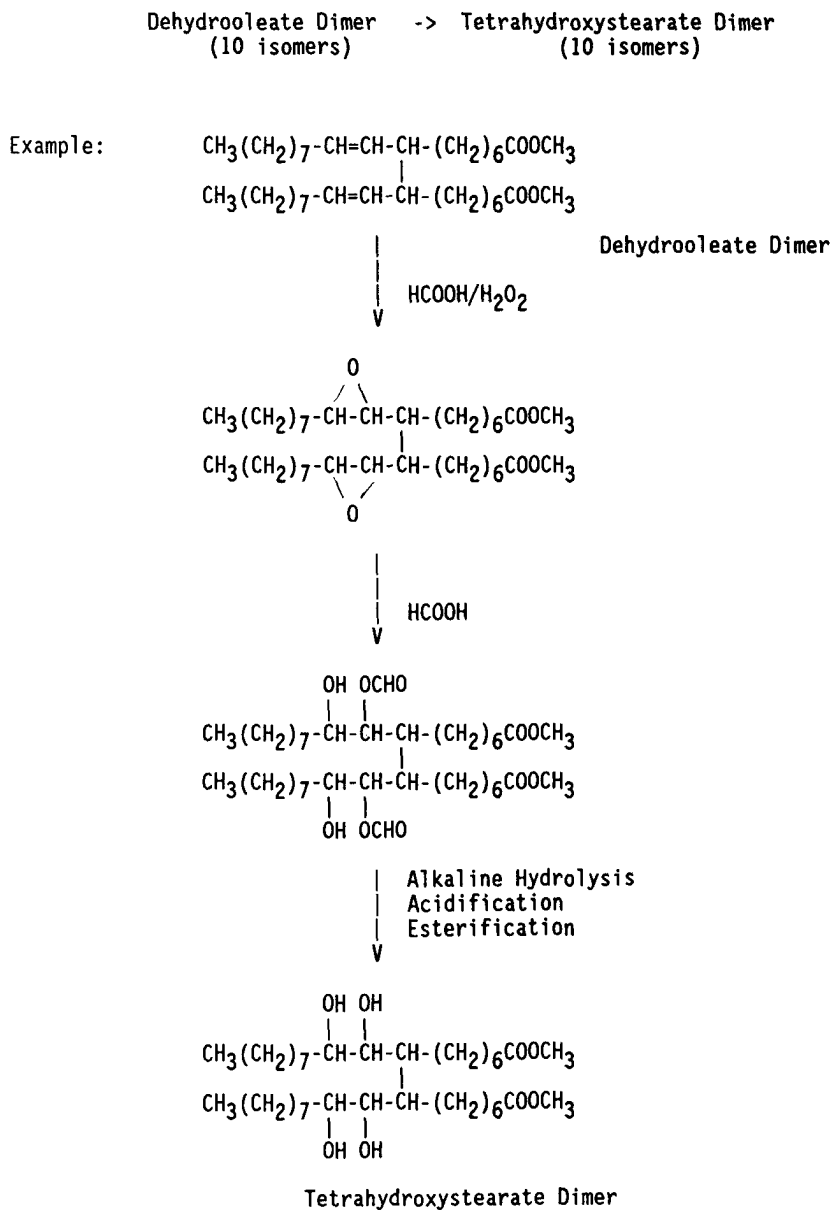

FIG. 1. Formation of the tetrahydroxystearate dimer (MW = 658).

TABLE 1

Infrared Absorption Spectral Data from the Tetrahydroxy, Dihydroxy, and Diketostearate Dimers

Frequency (cm^{-1})	Functional group	Assignment	Frequency (cm^{-1})	Functional group	Assignment
3445	Free OH	O-H stretch	1430-1440	$C-CH_3$	Asym. C-H bend
2915-2900	$-(CH_2)_n-$	Asym. C-H stretch	1365-1375	$C-CH_3$	Sym. C-H bend
2840-2860	$-(CH_2)_n-$	Sym. C-H stretch	1250		
	O		1200	O	
1735	$-C-O-OCH_3$	C=O stretch	1170	$-C-O-CH_3$	C-O stretch
	O		1095		
1720	CH_2-C-CH_2	C=O stretch	1020		
			935	all OH	O-H vibrations
1460	$-(CH_2)_n-$	Asym. C-H scissor	725-735	$-(CH_2)_n-$	C-H rock

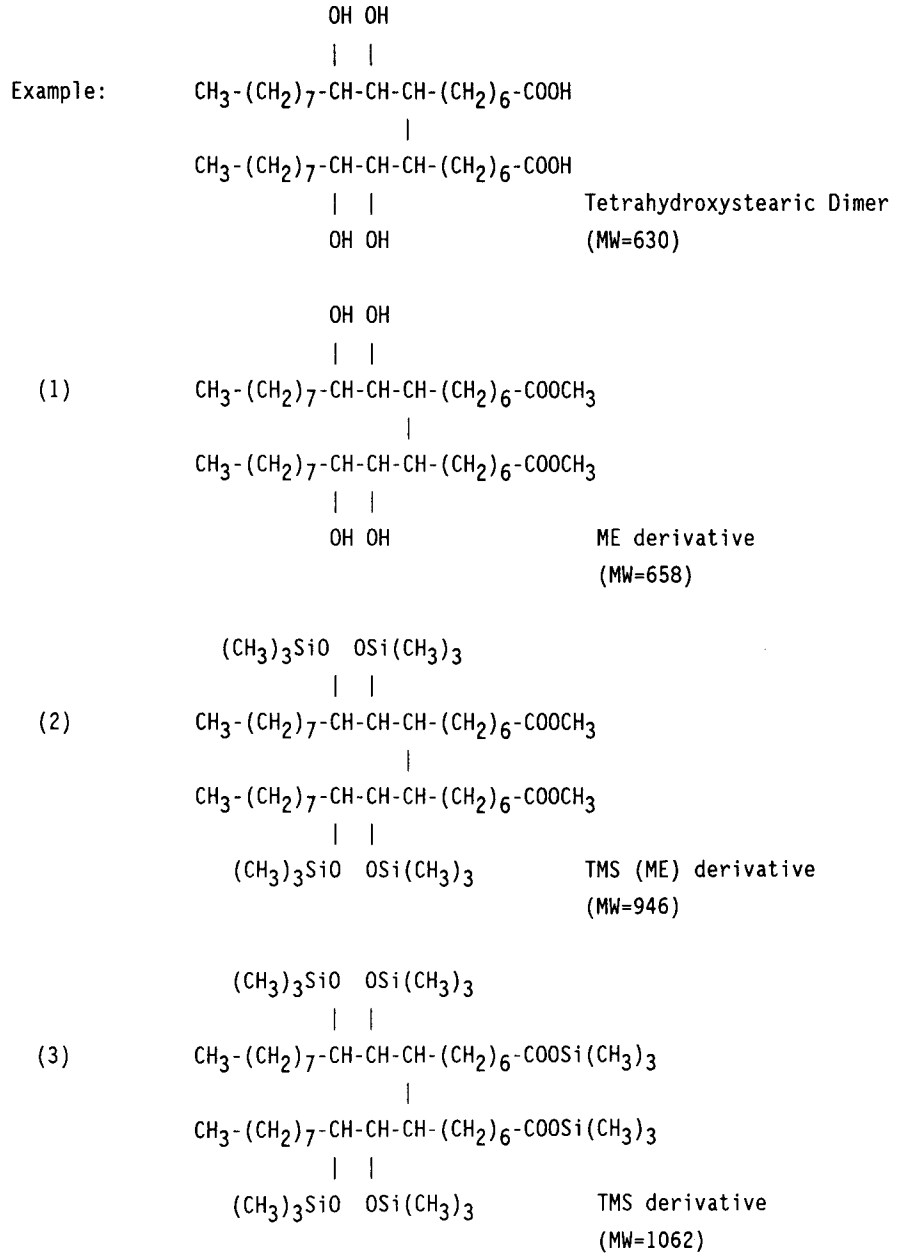

FIG. 2. Representative structures of the derivatives used in mass spectrometric studies of the tetrahydroxystearic dimer.

TABLE 2

Mass Spectral Data from the Tetrahydroxystearic Dimer as the Methyl Ester Derivative[a]

Fragment ions m/e (rel. abundance)	Assignment	Fragment ions m/e (rel. abundance)	Assignment
Electron ionization		Chemical ionization	
295(12.3)	M/2-2(OH)	641 (14.6)	[M-(OH)-H]+1
311(28.2)	M/2-(OH)-H	623(100.0)	[M-2(OH)-2H]+1
493(39.3)	M-4(OH)-C_7H_{15}+2H	622 (19.9)	M-2(OH)-2H
479(49.7)	M-4(OH)-C_8H_{17}+2H	604 (4.8)	M-3(OH)-3H
461(19.2)	M-4(OH)-(C_7H_{15}+CH_3OH)+2H	605 (43.2)	[M-3(OH)-3H]+1
417(16.4)	M-4(OH)-($C_{10}H_{21}$+2CH_3OH)+2H	586 (6.3)	M-4(OH)-4H
449(53.5)	M-4(OH)-$(CH_2)_6COOCH_3$+2H	587 (6.3)	[M-4(OH)-4H]+1
435(61.5)	M-4(OH)-$(CH_2)_7COOCH_3$+2H		

[a]Mechanism of formation and structure as in Fig. 1.

TABLE 3

Mass Spectral Data from the Tetrahydroxystearic Dimer as TMS of the Methyl Ester Derivative[a]

Fragment ions m/e (rel. abundance)	Assignment
Electron ionization	
539(14.5)	M-(4TMSO)-$(CH_2)_4COOCH_3$
525(15.4)	M-(4TMS)-$(CH_2)_5COOCH_3$
493(22.6)	M-4TMSO-C_7H_{15}+2H
479(29.0)	M-4TMSO-C_8H_{17}+2H
455 (1.5)	M-4TMSO-$(CH_3OH)_2$-C_5H_{11}
449(41.0)	M-4TMSOH-(C_8H_{17}+CH_3OH)
383(68.1)	M/2-90
Chemical ionization	
814 (8.2)	M-2($COOCH_3$)-CH_2
770 (24.4)	M-2($COOCH_3$)-$(CH_2)_4$-2H
696(100.0)	M-3TMS-OCH_3
637 (16.1)	M-3TMS-TMSO
605 (59.7)	M-3TMS-CH_3OH-TMSOH
590 (4.8)	M-4TMSO

[a]Structure of the TMS derivative of the methyl ester of the dimer in Fig. 2.

TABLE 4

Mass Spectral Data from the Tetrahydroxystearic Dimer as TMS Derivative[a]

Fragment ions m/e (rel. abundance)	Assignment
Electron ionization	
493(32.5)	M-[(2TMSO+$CH_3(CH_2)_x$-CH] +[(2TMSO+$CH_3(CH_2)_y$-CH_4] x=7,6; y=4,5
479(37.1)	M-[(2TMSO+$CH_3(CH_2)_x$-CH] +[(2TMSO+$CH_3(CH_2)_y$-CH_4] x=7,6; y=5,6
461(21.9)	M-[(2TMSO+$CH_3(CH_2)_x$-CH] +[(2TMSO+$CH_3(CH_2)_y$-CH_4)] x=7; y=6
435(56.3)	M-[2TMSO+$CH_3(CH_2)_x$-CH] +[2TMSO+$(CH_2)_y$COOTMS] x=7,6; y=2,3
449 (5.1)	M-[2TMSO+$CH_3(CH_2)_x$-CH] +[2TMSO+$(CH_2)_y$COOTMS] x=7,6; y=1,2
441 (6.3)	M/2-TMSOH
383(96.4)	M/2-2TMS-2H
Chemical ionization	
930 (5.1)	M-TMSOC(OH)=CH_2
886 (34.3)	M-2TMSO+2H
or	M-[TMSOC(OH)=CH_2]-$(CH_2)_2$-CH_4
814 (21.0)	M-[TMSOCO$(CH_2)_2$-CH_4]-TMSO
812 (56.4)	M-[TMSOCO$(CH_2)_3$]-TMSO
770 (14.1)	M-4TMSO
722 (27.7)	M-[TMSOCO$(CH_2)_2$-CH_4]-2TMSO-H
696 (76.6)	M-2[TMSOC(OH)=CH_2]-$(CH_2)_5$-2(CH_4)
652 (64.4)	M-2[TMSOC(OH)=CH_2]-2TMS
624 (22.1)	M-2[TMSOC(OH)=CH_2]-2(CH_2)-2TMS
605(100.0)	M/2+TMS+H

[a]Structure of the TMS derivative of the dimers in Fig. 2.

OH
|

m/e 173 and 187 which represent $(-CH(CH_2)_xCOOCH_3)^+$, x = 6,7) as well as the ions representing the loss of CH_3OH from these ions at m/e 155 and 141 were observed, and indicate the presence of isomeric tetrahydroxystearate dimers.

The chemical ionization (CI) mass spectrum gave peaks corresponding to ions resulting from the loss of two or three H_2O molecules from the tetrahydroxystearate dimer. For the methyl 9,10 dihydroxystearate employed as a model compound, two characteristic ions at $[M-(OH)]^+$ were obtained. Equivalent ions were not seen in the CI spectrum of the dimer.

The interpretation of spectral data from the TMS (ME) derivative of tetrahydroxystearate dimer is given in Table 3. The TMS derivatives of dihydroxy acids cleave between the substituted carbon atoms yielding abundant characteristic fragments, and are useful for the determination of various isomeric structures (19-21). The molecular ions were not observed but were represented by ions at $[M-CH_3]^+$ and $[M-OCH_3]^+$. In the chemical ionization spectrum the base peak is at M-TMSO as was observed for the TMS derivative of the methyl 9,10-dihydroxystearate.

OTMS
|

Fragment ions at m/e 215 $[CH_3-(CH_2)_7-CH-]^+$, 259

OTMS OTMS
| |

$[-CH-(CH_2)_7COOTMS]^+$, 201 $(CH_3-(CH_2)_6CH)^+$, and 245

OTMS
|

$(-CH-(CH_2)_7COOTMS)^+$ resulting from the cleavage between the two substituted carbon atoms are present and provide evidence for the presence of tetrahydroxystearate dimer isomers. However, ions which would result from the loss of the above ions from the molecular ion were absent. This is in accordance with the results observed by others (22) for the TMS derivatives of polyunsaturated fatty acids where ions which represented the loss of a TMS fragment from the molecular ion are not present. Similarly, ions of the general formulas $[X + 73]^+$, $[X + 102]^+$, $[M - 90)]^+$, $[M - (X - 102)]^+$, $[M - (X - 73)]^+$, and $[M - (X - 102 + 90)]^+$, where X is the fragmented ion, M the molecular weight of the dimer, and m/e 73, 90, 102 representing $-[Si(CH_3)_3]^+$, $[HOSi(CH_3)_3]^+$, and $[CH-OSi(CH_3)_3]^+$, respectively, were not observed in the spectrum. The ion at m/e 383, $[M/2-TMSOH]^+$, was the base peak. Other ions present in the spectrum were explained by elimination of the TMSO groups and loss of either an alkyl or ester group from the dimer with or without hydrogen rearrangement. The CI spectrum showed no characteristic peaks for a dimer of MW=946, but rather various high molecular weight fragment ions related to the molecular ion as explained in Table 3.

The mass spectral data from the TMS derivative of the tetrahydroxystearic acid dimer are presented in Table 4. The various ions may be explained by the elimination of the TMSO groups plus various alkyl or ester fragments. The ion at m/e 383 appears to have resulted from $[M/2-2TMS-2H]^+$.

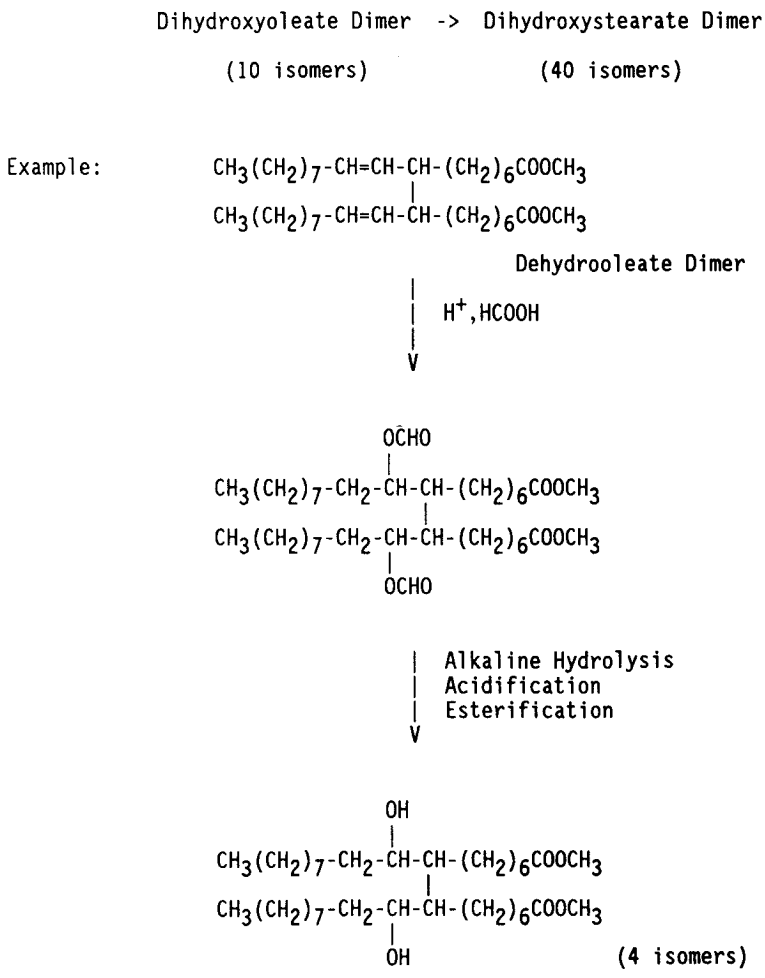

FIG. 3. Formation of dihydroxystearate dimer (MW=626).

The CI spectrum of this compound was very useful. Several high molecular weight ions were present. The ion at m/e 930 directly relates to the molecular ion M=1062 by loss of a fragment due to the McLafferty rearrangement for a TMS ester. The base peak at m/e 605 was explained as the M/2 ion plus a TMS group commonly found in polyTMS derivatives. Various other ions can be explained mainly by the loss of TMSO groups, and other fragments due to the McLafferty rearrangement plus the loss of CH_2 groups and methane.

The formation of the dihydroxystearic dimers from the dehydrodimers of methyl oleate is illustrated in Figure 3. Formic acid was added to the dehydrodimer of methyl oleate in the presence of strong acetic acid using method described by Knight *et al.* (16). This resulted in the formation of four isomers of formoxystearate dimer for each isomer of the dehydrooleate dimer which, by alkaline hydrolysis, acidification and esterification would give four isomeric dihydroxystearate dimer structures for each isomer of dehydrooleate dimer.

GLC analysis of the methyl ester of the final product from the synthesis of the dihydroxystearic dimer gave a main peak at RRT=2.64, with a minor peak at RRT=2.23, indicating the presence of some unreacted dimer. Similarly, GLC analysis of the TMS derivative gave a main peak at RRT=2.50, with a minor peak at the retention time of the TMS derivative of the dihydrodimer of oleic acid, which was subsequently removed by LC. The purity of the dihydroxystearic dimer was assured as determined by GLC and TLC. The R_f value for the dihydroxystearate dimer was 0.25.

The IR absorption spectrum is presented in Table 1. The presence of strong peaks at 3445 and 935 cm^{-1}, which are representative of free hydroxyl groups and of all hydroxyl groups in the molecules provided evidence for the presence the hydroxyl groups in the molecule.

The tentative interpretation of the mass spectral data obtained for the methyl ester, the TMS derivative of the methyl ester, and the TMS derivative of the dihydroxystearic acid dimer (Fig. 4) are represented in Tables 5–7.

The methyl ester of the dihydroxystearate dimer showed no molecular ion or peak at m/e = $[M-(H_2O+CH_3OH)]^+$ as expected. Instead, an ion at m/e 590 which resulted from the molecular ion by the loss of two

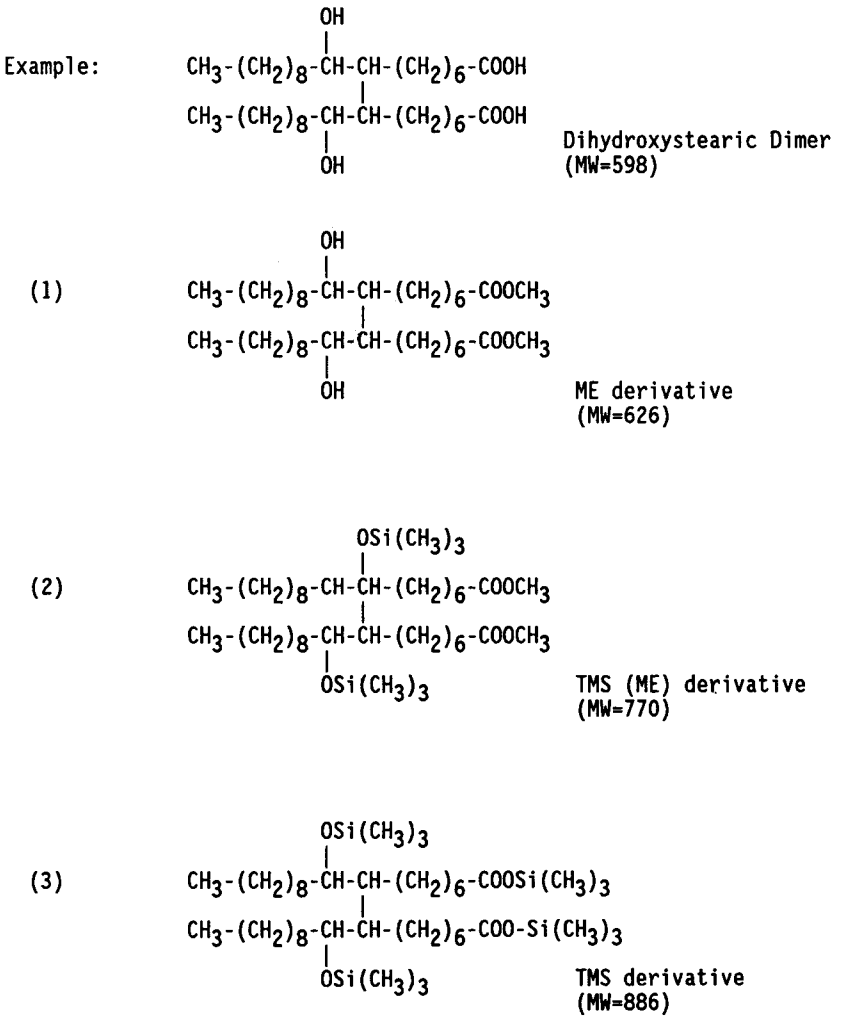

FIG. 4. Representative structures of the derivatives used in mass spectrometric studies of the dihydroxystearic dimer.

TABLE 5

Mass Spectral Data from the Dihydroxystearic Dimer as Methyl Ester Derivative[a]

Fragment ions m/e (rel. abundance)	Assignment
Electron ionization	
590 (5.3)	M-2(OH)-2H
559 (10.9)	M-2(OH)-OCH_3-2H
491 (51.5)	M-2(OH)-C_7H_{15}-2H
477 (65.1)	M-2(OH)-C_8H_{17}-2H
447 (61.7)	M-2(OH)-$(CH_2)_6COOCH_3$-2H
433(100.0)	M-2(OH)-$(CH_2)_7COOCH_3$-2H
419 (51.0)	M-2(OH)-$(C_{10}H_{20}+CH_3O)$-2H
Chemical ionization	
591(100.0)	M-2(OH+H)+1
559 (25.6)	M-2(OH+H)-31
619 18.5)	M-2(OH+H)+29

[a]Mechanism of formation and structure as in Fig. 3.

TABLE 6

Mass Spectral Data from the Dihydroxystearic Dimer as TMS of the Methyl Ester Derivative[a]

Fragment ions m/e (rel. abundance	Assignment
Electron ionization	
590 (4.9)	M-2TMSO
559 (7.8)	M-2TMSO-OCH_3
491 (38.5)	M-2TMSO-2H-$(CH_2)_6CH_3$
477 (64.4)	M-2TMSO-2H-$(CH_2)_7CH_3$
447 (58.4)	M-2TMSO-2H$(CH_2)_6COOCH_3$
433(100.0)	M-2TMSO-2H-$(CH_2)_7COOCH_3$
Chemical ionization	
591(100.0)	M-(2TMSO-2H)+1
619 (18.8)	M-(2TMSO-2H)+29
631 (3.5)	M-(2TMSO-2H)+41
575 (5.9)	M-(2TMSO-2H)-15
559 (28.5)	M-(2TMSO-2H)-31

[a]Structure of the TMS derivative of the methyl ester of the dimer in Fig. 4.

TABLE 7

Mass Spectral Data from the Dihydroxystearic Dimer as TMS Derivative[a]

Fragment ions m/e (rel. abundance)	Assignment
Electron ionization	
593(26.5)	M-2(COOTMS)-$CH_3(CH_2)_2$-CH_4
517 (6.0)	M-2(COOTMS)-TMSO-CH_2-2(CH_4)
505(71.4)	M-2(COOTMS)-TMSO-3(CH_2)-CH_4
491(80.1)	M-2(COOTMS)-TMSO-4(CH_2)-CH_4
477(20.8)	M-2(COOTMS)-TMSO-5(CH_2)-CH_4
355(19.3)	M/2-TMS
Chemical ionization	
708(21.8)	M-2TMSO
652(40.8)	M-2(COOTMS)
or	M-TMSO-$(CH_2)_2$COOTMS
591(15.9)	M-2(TMSO)-COOTMS
535(13.1)	M-2TMSO-$(CH_2)_4$COOTMS
443(11.8)	M/2
355(31.0)	M/2-TMSO

[a]Structure of the TMS derivative of the dimer in Fig. 4.

water molecules and abstraction of two hydrogen atoms is present (Table 5). Characteristic ions resulting from the cleavage of the substituent chains on either side of the carbon containing the OH group are present as very weak ions. The major ions shown are explained by subtraction of the two hydroxyl groups, loss of alkyl or ester fragment, and hydrogen atoms. The CI spectrum gave a molecular ion at m/e 590 which was obtained from the molecular weight by subtraction of two H_2O molecules. This is similar to the loss of H_2O from methyl-12-hydroxystearate during CI which gave an ion at $[M-H_2O]^+$.

In Table 6, the mass spectral data from the TMS derivative of the methyl ester of the dihydroxystearic dimer are presented. The molecular ion was absent, but a peak at m/e 590, resulting from the molecular ion by subtraction of the TMSO groups was present in the EI spectrum. Ions corresponding to fragments resulted from cleavage of the substituents at either side of the carbon atoms of the OTMS group are present but weak. The ions observed may be explained by subtraction of the two TMSO groups and loss of alkyl or ester fragments with hydrogen rearrangement from the molecular ion. In the CI spectrum the molecular ion obtained at m/e 590 by loss of two TMSOH groups was analogous to the loss of TMS OH groups from the TMS derivative of methyl-12-hydrostearate.

The mass spectral data from the TMS derivative of the dihydroxystearic dimer are shown in Table 7. In the EI spectrum, the intensity of the fragments which resulted from cleavage at either side of the carbon containing the OTMS group was low. The various ions present in the spectrum were explained mainly by loss from the molecule of TMSO, COOTMS, and various alkyl groups. The ion at 355 was explained as the $[M/2-TMSO]^+$ ion and provided evidence for the molecular weight assignment. The CI spectrum proved to be very useful. An ion at m/e 708 represents the molecular ion with loss of TMSO groups. In comparison the ion representing $[M-TMSO]^+$ was the base peak in the CI spectrum of the 1-TMSO derivative of methyl-12-hydroxystearate. The ions at m/e 443 and 355 were the $[M/2]^+$ and $[M/2-TMSO]^+$ peaks, respectively, and provided further evidence of the molecular weight determination of the dimer derivative. Other ions present in the spectrum may be explained as shown in Table 7 by substraction of TMSO, COOTMS, methane, and various alkyl groups from the molecular ion (22).

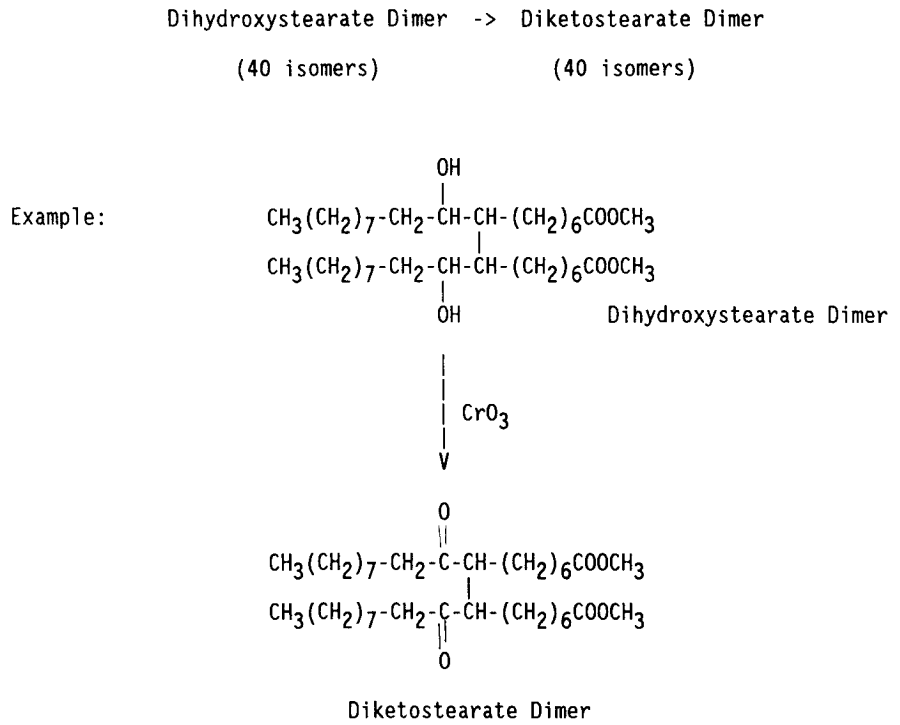

FIG. 5. Formation of diketostearate dimer (MW=622).

TABLE 8

Mass Spectral Data from the Diketostearic Dimer as Methyl Ester Derivative[a]

Fragment ions m/e (rel. abundance)	Assignment
Electron ionization	
622 (1.0)	M
311(27.6)	M/2
279 (12.3)	M/2-OCH_3
604 (40.3)	M-H_2O
573 (28.1)	M-H_2O-OCH_3
541 (6.1)	M-H_2O-OCH_3-CH_3OH
507 (24.1)	M-$CH_3(CH_2)_7$-OCH_3
492 (73.9)	M-$CH_3(CH_2)_6$-OCH_3
462 (89.9)	M-2($COOCH_3$)-$(CH_2)_3$
448 (89.9)	M-2($COOCH_3$)-$(CH_2)_4$
434 (17.7)	M-2($COOCH_3$)-$(CH_2)_5$
419 (42.6)	M- ($COOCH_3$)-$(CH_2)_5CH_3$
325 (34.2)	M-[$(CH_3(CH_2)_xC(=O)-)+CH_3(CH_2)_yC(OH)=CH_2$] x=6,7,8; y=8,7,6
349 (12.1)	M-[$CH_2=COOCH_3+CH_2=C(OH)-(CH_2)_7COOCH_3$]
Chemical ionization	
623 (37.1)	M+1
651 (11.0)	M+29
663 (2.9)	M+41
605(100.0)	M-(OH)

[a]Mechanism of formation and structure as in Fig. 5.

Finally, ions at m/e 201, 215, 229, 303, 317, and 331 provided evidence for the presence of all positional isomers of dihydroxystearic acid since these ions represent the various fragments containing the TMSO group resulting from cleavage at either side of substituted carbon atoms.

Diketostearate dimers were prepared by CrO_3 oxidation of the dihydroxystearate dimer as described by Ross *et al.* (17) (Fig. 5).

GLC analysis of the methyl ester gave one main peak at RRT=3.36. TLC analysis gave an R_f value of 0.24. The 1R absorption spectrum (Table 1) was similar to that of methyl oleate except for the peak at 1720 cm^{-1} due to

$-CH_2-\overset{O}{\overset{\|}{C}}-CH_2-$ present in the molecule.

The EI and CI mass spectral data of the diketostearate dimer are presented in Table 8.

The EI spectrum of the diketostearate dimer showed a weak molecular ion at m/e 622. Ions at m/e 604 and 573 represent loss from the molecular ion of H_2O and H_2O plus OCH_3, respectively. The $[M/2]^+$ peak was observed at m/e 311, along with the peak at m/e 279 which represents the loss of CH_3O from the $[M/2]^+$ peak. Peaks at m/e 127, 141, 155, 171, 185, and 199 of two general ion series—

$([CH_3-(CH_2)_xC(=O)-]^+$, x=6,7,8) and $([-C(=O)(CH_2)_yCOOCH_3]^+$, y=6,7,8)—which resulted from cleavage of the chain at either side of the carbon atom of the carbonyl group were present and denote the presence of diketostearate dimer isomers.

Similarly, the presence of ions at m/e 142, 156, 170, 187, 200, and 214 of the general ion series

$([CH_3(CH_2)_xC(OH)=CH_2]^+$, x=6,7,8) and

$([-CH_2=C(OH)-(CH_{2y}COOCH_3]^+$, x=6,7,8),

which result from the McLafferty rearrangement of either hydrogen to the carbonyl function in the chain provided additional evidence for the presence of the various diketostearate isomeric dimers (23). The various ions observed in the mass spectrum may be explained mainly by loss from the molecular ion of $-COOCH_3$ and various alkyl groups (Table 8).

Ions resulting from loss from the molecular ion of the various fragment ions were seen only for the ions at m/e 325 and 349. The CI spectrum showed the characteristic peaks for molecular weight 622. The base peak at m/e 605 was explained as an ion resulting from loss of a hydroxyl group from the molecular ion. This ion representing an analogous loss was not present in the CI spectrum of methyl-12-ketostearate.

ACKNOWLEDGMENT

Partial support from the University of Illinois Agricultural Experimental Station is gratefully acknowledged.

REFERENCES

1. Perkins, E.G. and F.A. Kummerow, *J. Nutrition 68*:101 (1959).
2. Sahasrabudhe, M.R. and I.G. Farn, *J. Am. Oil Chem. Soc. 41*:264 (1964).
3. Firestone, D., W. Hozwitz, L. Friedman and G.M. Shue, *Ibid. 38*:253 (1961).
4. Ohfuji, T. and T. Kaneda, *Lipids 8*:353 (1973).
5. Zeman, A. and Scharmann, H., *Fette Seifen Anstrichm. 71*:957 (1969).
6. Perrin, J.L., P. Perfetti, C. Dimitriades and M. Naudet, *Rev. Franc. Corps Gras. 32*:151 (1985).
7. Perrin, J.L., P. Perfetti and M. Naudet, *Ibid. 32*:205 (1985).
8. Gere, A., *Ibid. 31*:437 (1984).
9. Gere, A., J.L. Sébédió and A. Grandgirard, *Fette Seifen Anstrichm. 85*:359 (1985).
10. Kypranycz, D.B., M.A. Amer and B.E. Baker, *J. Am. Oil Chem. Soc. 63*:332 (1986).
11. Michael, W.R., J.C. Alexander and N.R. Artman, *Lipids 1*:353 (1966).
12. Perkins, E.G. and L.R. Wantland, *J. Am. Oil Chem. Soc. 50*:459 (1973).
13. Chang, S.S., R.J. Peterson and C.T. Ho, *Ibid. 55*:718 (1978).
14. Paschke, R.F., L.E. Peterson, S.A. Harrison and D.H. Wheeler, *Ibid. 41*:56 (1964).
15. Swern, D., J.T. Scanlan and G.B. Dickel, *Organic Syntheses*, Vol. IV, p. 317 (1963).
16. Knight, H.B., R.E. Koos and D. Swern, *J. Am. Oil Chem. Soc. 31*:1 (1954).
17. Ross, J., A.I. Gebhart and J.F. Gerecht, *Ibid. 71*:282 (1949).
18. American Oil Chemists' Society, *Official and Tentative Methods*, Vol. 1, 3rd edition, AOCS, Champaign, IL, 1981. Method Ce 1-62.
19. Capella, P. and C.M. Zorzut, *Anal. Chem. 40*:1458 (1968).
20. Argoudelis, C.J. and E.G. Perkins, *Lipids 3*:379 (1968).
21. Argoudelis, C.J. and E.G. Perkins, *Ibid. 4*:619 (1969).
22. Mccloskey, L.A., in *Topics of Lipid Chemistry*, edited by F.D. Gunstone, John Wiley & Sons, Inc., New York, NY, pp. 369-437 (1970).
23. Ryhage, R. and E. Stenhagen, *J. Lipid Research 1*:361 (1960).

[Received June 13, 1988; accepted March 3, 1989]
[J5484]