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Isotope Labeling Studies on the Electron Impact Mass Spectral Fragmentation Patterns of Chloropropanol Acetates

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ABSTRACT: Chloropropanol (CP) esters are part of an emerging group of process-induced toxicants that are considered as potential health hazards particularly in palm oil. Mass spectrometry-based methodologies for identification of CP esters in food are critical in overcoming the challenges associated with direct detection methods. In the present study, a convenient strategy was employed to generate all possible CP acetates through reacting acetic anhydride with either glycerol in the presence of a chloride source or the corresponding CPs, such as 3-chloro-, 1,3-dichloro-, 2-chloro-, and 1,2-dichloropropanols, allowing for the identification of the individual CP acetates and assignment of their mass spectral fragmentations. Mass spectral fragmentations were confirmed through the use of the isotopic signature of chlorine in addition to the isotope labeling experiments performed using isotopically labeled precursors, such as [^{13}C-U$_3$] glycerol, [^{13}C-U$_4$] acetic anhydride, [^{13}C-2,2′] acetic anhydride, and [d$_5$] 3-monochloropropane-1,2-diol (3-MCPD) as reactants. Such studies have indicated that all CP esters can undergo two general fragmentations under electron impact (EI) conditions, one generating the acylium ion at m/z 45 and the other generating a chlorinated cyclic acyloxonium ion at m/z 135.6. Considering the fact that such ions can also be generated from any fatty acid containing CP esters after undergoing McLafferty rearrangement, the ion at m/z 135.6 can therefore be considered as a universal marker for the presence of CP esters undergoing EI fragmentation. Furthermore, these studies have also indicated the formation of ions characteristic of CP diesters, monoesters, and dichloro esters.

KEYWORDS: *Chloropropanol acetates, EIMS spectra, isotope labeling, acyloxonium ions, CP esters, McLafferty rearrangement*

INTRODUCTION

Chloropropanol (CP) esters (Figure 1) are part of an emerging group of process-induced toxicants that are considered as potential health hazards particularly in palm oil or food products rich in palm oil.[1] Originating primarily from the deodorization stage of oil refining, CP esters require high temperatures and acid catalysis for their generation. Their formation mechanism has been reviewed recently,[1−3] and the source of chlorine in the crude palm oil has been identified.[4] Considering the number and position of naturally occurring fatty acids on the glycerol backbone carrying the chlorine, the resulting structural diversity and complexity of such CP esters poses a formidable challenge for their direct analysis.[5,6] Because of the difficulty in analyzing such complex mixtures, the samples are normally hydrolyzed before analysis to convert all CP esters into their corresponding CPs, which, in turn, are commonly derivatized before further quantification using various gas chromatography (GC)- or liquid chromatography (LC)-based methodologies.[7−10] This approach precludes the ability to profile all CP esters in edible oils.[5] Direct detection of CP esters are presently avoided,[9] necessitating development of an alternative approach.[7] Progress toward developing methodologies for the direct and simultaneous quantitative analysis of individual CP esters requires detailed understanding of their characteristic and unique fragmentation pathways to determine diagnostic ions for application in any mass spectrometry (MS)-based methodology. Gardner and colleagues[11] were among the first to use characteristic mass spectral ions of specific CP diesters identified earlier by Davidek et al.[12] to confirm their presence in the adulterated Spanish olive oil samples. Nevertheless, there is limited data in the literature on the detailed mass spectral fragmentation patterns not only of CP esters but also of triacylglycerides (TAGs) in general.[5] Velíšek et al.[13] characterized the CP esters resulting from the reaction of triacetin and tributyrin in the presence of HCl through GC/MS. Meanwhile, Kraft and colleagues[14] synthesized CP esters according to procedures published by Brachwitz et al.,[15] characterizing their mass spectra containing higher fatty acids, such as lauric, palmitic, stearic, and behenic acids. The following year, Davídek et al.[12] investigated the chlorination of triolein, tripalmitin, and tristearin, tentatively identifying the resulting CP esters through infrared (IR), MS, and nuclear magnetic resonance (NMR). Recent accessibility to commercial CP ester standards has promoted the development of direct methods using liquid chromatography−tandem mass spectrometry (LC−MS/MS) techniques.[16,17] Although some mass spectra of CP esters are reported in the literature, nevertheless, the structure of proposed ion fragments is not confirmed through isotope labeling data and no systematic studies have been conducted to characterize a general mass spectral fragmentation pattern. In the present study, a convenient strategy was employed to generate all possible CP acetates through reacting acetic anhydride with glycerol and the corresponding CPs, allowing for the identification of the individual CP acetates. This approach was further extended to

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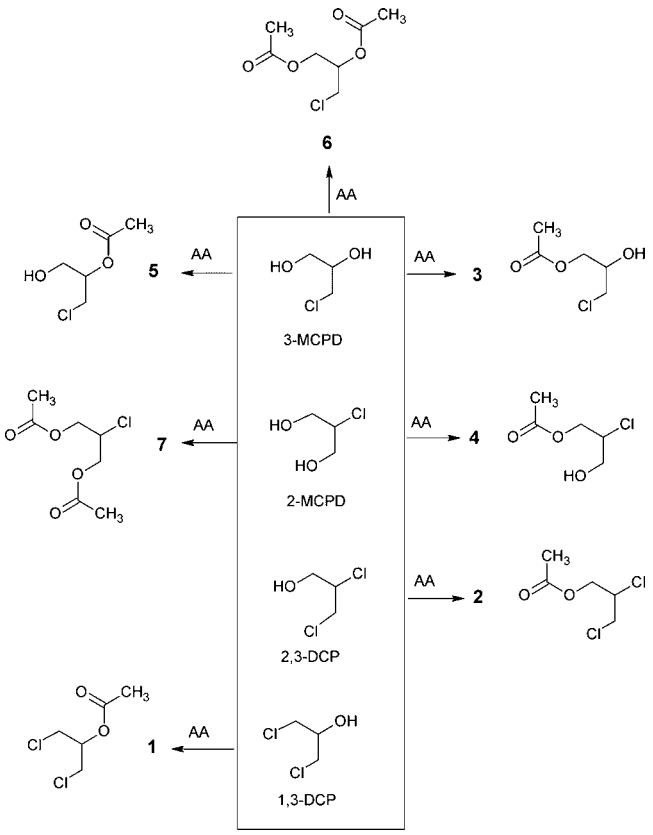

Figure 1. Generation of mono- and dichloropropanol esters from their respective precursors in the presence of acetic anhydride. AA, acetic anhydride; 3-MCPD, 3-monochloropropanediol; 2-MCPD, 2-monochloropropanediol; 2,3-DCP, 2,3-dichloro-1-propanol; and 1,3-DCP, 1,3-dichloro-2-propanol.

include other short-, medium-, and long-chain fatty acid anhydrides (will be published elsewhere). Identification of the fundamental fragmentation patterns of the mass spectra of CP esters facilitates development of specific assays for their detection through MS-based methodologies. While the electron impact (EI) mass spectrometric analysis of short-chain CP esters may not reveal the fragmentation pathways involved in the longer fatty acid carbon chains, nevertheless, glycerol-moiety-based fragments responsible for carrying the chlorine atom(s) could provide valuable insight into the identification of diagnostic ions. In addition to the isotopic signature of chlorine, isotopically labeled precursors were used to identify the participation of the carbon atoms within the individual mass fragments. Furthermore, longer chain fatty acids undergoing McLafferty rearrangement can be converted into acetate esters under EI fragmentation; thus, the commonly encountered McLafferty rearrangement products in the EI spectra of lipids further underline the importance of studying acetate esters.

MATERIALS AND METHODS

3-Monochloro-1,2-propanediol (98%) and 2,3-dichloro-1-propanol (2,3-DCP) (>97.0%) were purchased from Sigma-Aldrich (St. Louis, MO). 1,3-Dichloro-2-propanol (1,3-DCP) (98%) was purchased from ACP Chemicals, Inc. (Montreal, Quebec, Canada). Acetic anhydride (≥97%) was obtained from Anachemia (Lachine, Quebec, Canada). 2-Chloro-1,3-propanediol (2-MCPD) (98%) and 3-chloro-1,2-propanediol (propane-d_5, 98%) were purchased from Toronto Research Chemicals (Ontario, Canada). Acetic anhydride (1,1′- and 2,2′-$^{13}C_4$, 99%), acetic anhydride (2,2′-$^{13}C_2$, 99%), and glycerol ($^{13}C_3$, 99%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). All chemicals were used without further purification. The *rac*-1,2-bis-acetoyl-3-chloropropanediol was synthesized according to Rahn and Yaylayan.[18]

***In Situ* Generation of CP Acetates Using Pyrolysis–Gas Chromatography–Mass Spectrometry (Py–GC/MS).** Varian CP-3800 GC equipped with a sample pre-concentration trap (SPT) filled with Tenax GR was coupled to a Varian Saturn 2000 ion-trap mass spectrometer (Varian, Walnut Creek, CA). The pyrolysis unit included a valved interface (CDS 1500), which was installed onto the GC injection port and connected to a CDS Pyroprobe 2000 unit (CDS Analytical, Oxford, PA). In all experiments (see Table 1), the triacetin/glycine hydrochloride model mixtures (1 mg, 1:2 molar ratio) were introduced inside the quartz tube (0.3 mm thickness), plugged with quartz wool, and inserted into the coil probe. The temperature of the pyroprobe interface was set at 250 °C. Model systems were pyrolyzed at 250 °C with a total heating time of 20 s. The analytes generated during pyrolysis were concentrated using a SPT at 50 °C and released onto the column after 5 min, under a steady stream of helium. The initial temperature of the column was set at −5 °C for 5 min and then increased to 50 °C at a rate of 50 °C/min; immediately, the temperature was further increased to 280 °C at a rate of 8 °C/min and kept at 280 °C for 6 min. A constant flow of 1.5 mL/min was used during analysis. The MS interface temperature was set to 250 °C, and the ion source temperature was 175 °C. The ionization voltage was 70 eV, and the electron multiplier was set at 1500 V. The mass range analyzed was 20–650 amu. The column was a fused silica DB-5 MS column (50 m length, 0.2 mm inner diameter, and 0.33 μm film thickness; J&W Scientific, Folsom, CA). The identity and purity of the chromatographic peaks were determined using National Institute of Standards and Technology (NIST) Automated Mass Spectral Deconvolution and Identification System (AMDIS), version 2.1 (http://chemdata.nist.gov/mass-spc/amdis/).

Table 1. Model Systems Used To Generate Labeled and Unlabeled CP Acetates

triacetin
triacetin + glycine hydrochloride
glycerol + acetic anhydride
glycerol + acetic anhydride + glycine hydrochloride
[^{13}C-U_3] glycerol + acetic anhydride + glycine hydrochloride
glycerol + [^{13}C-2,2′] acetic anhydride + glycine hydrochloride
glycerol + [^{13}C-U_4] acetic anhydride + glycine hydrochloride
3-monochloropropane-1,2-diol + acetic anhydride
3-monochloropropane-1,2-diol + [^{13}C-2,2′] acetic anhydride
3-monochloropropane-1,2-diol + [^{13}C-U_4] acetic anhydride
2-monochloropropane-1,2-diol + acetic anhydride
2-monochloropropane-1,2-diol + [^{13}C-2,2′] acetic anhydride
2-monochloropropane-1,2-diol + [^{13}C-U_4] acetic anhydride
1,3-dichloropropanol + acetic anhydride
1,3-dichloropropanol + [^{13}C-2,2′] acetic anhydride
1,3-dichloropropanol + + [^{13}C-U_4] acetic anhydride
2,3-dichloropropanol + acetic anhydride
2,3-dichloropropanol + [^{13}C-2,2′] acetic anhydride
2,3-dichloropropanol + + [^{13}C-U_4] acetic anhydride
additional model systems
3-monochloropropanediol-1,2-diacetate
[d_5] 3-monochloropropane-1,2-diol + acetic anhydride

***In Situ* Generation of Isotopically Labeled CP Acetates Using Py–GC/MS.** The above procedure was used, except the model systems were replaced with that of glycerol/acetic anhydride and their isotopically labeled counterparts [acetic anhydride (1,1′- and 2,2′-$^{13}C_4$) or acetic anhydride (2,2′-$^{13}C_2$) and [$^{13}C_3$]glycerol] as well as [d_5] 3-monochloropropane-1,2-diol (see Table 1). The mixtures contained one labeled component at a time. The chloride source was

glycine hydrochloride, which was separated from the model system mixture in the quartz tube by a piece of quartz wool.

RESULTS AND DISCUSSION

CP esters (Figure 1) constitute a complex mixture of monochloro and dichloro isomers and stereoisomers,[6] numbering up to 12 structures assuming the involvement of only one type of fatty acid in their generation.[19] However, this number can dramatically increase if more than one fatty acid is involved.[19] Because of the difficulty in analyzing such complex mixtures, as mentioned above, the samples are normally hydrolyzed before analysis to convert all structures into their corresponding free CPs. This approach, however, eliminates the ability to profile different isomers of CP esters.[5] Nagy and colleagues[4] have tried to address this limitation by identifying the chlorine content by mass defect differences. The mass defect difference between two isotopes of an atom remains constant and, therefore, serves as a compositional signature of the halogen content within mass spectra. Although this approach may facilitate identification of a broad range of chlorinated compounds, it does not distinguish between CP esters and other chlorinated analytes, precluding proper risk

Table 2. Retention Times and EI Mass Spectra[a] of CP Acetates Shown in Figure 1

compound	m/z (abundance)	retention time (min)
1	171 (4), **135** (1), 110 (2), 77 (4), 75 (13), 61 (4), 49 (4), 44 (3), **43** (100), 39 (5)	17.381
2	171 (1), **135** (0.4), 112 (24), 110 (4), 77 (3), 75 (10), 62 (3), 61 (7), 49 (5), 44 (2), **43** (100), 39 (5)	17.899
3	153 (3), **135** (7), 103 (8), 74 (6), 61(3), 57 (3), 49 (3), 44 (5), **43** (100), 42 (5)	18.323
4	153 (1), **135** (4), 103 (1), 92 (3), 86 (6), 85 (3), 74 (2), 64 (11), 62 (31), 61 (9), 57 (8), **43** (100), 42 (8)	17.971
5	153 (3), **135** (5), 103 (14), 74 (13), 61 (2), 49 (4), 44 (7), **43** (100), 42 (6), 39 (2)	17.745
6	145 (3), 137 (14), 136 (4), **135** (45), 103 (18), 101 (4), 99 (6), **43** (100), 42 (6), 39 (4)	20.273
7	137 (20), 136 (5), **135** (58), 115 (4), 99 (22), 97 (2), 44 (2), **43** (100), 39 (4), 27 (2)	20.435
6[b]	145 (3), 137 (14), 136 (3), **135** (41), 103 (18), 101 (3), 99 (8), **43** (100), 39 (3), 36 (3)	20.262

[a]Bold masses represent the base peak and the characteristic ion at m/z 135. [b]Synthetic 3-MCPD diacetate.

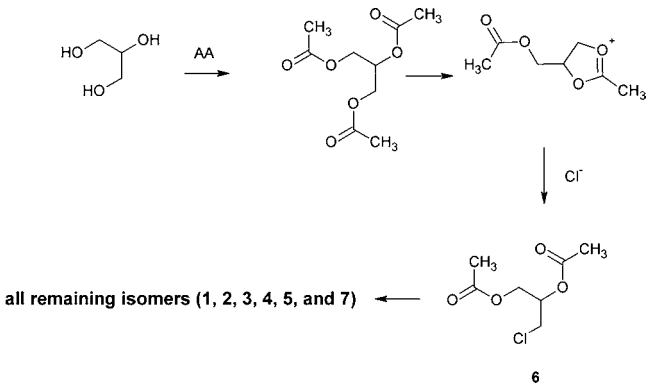

Figure 3. Proposed reaction pathway for the generation of CP acetates from triacetin or glycerol/acetic anhydride model systems in the presence of a chlorine source (see also Figures 5–7). AA = acetic anhydride.

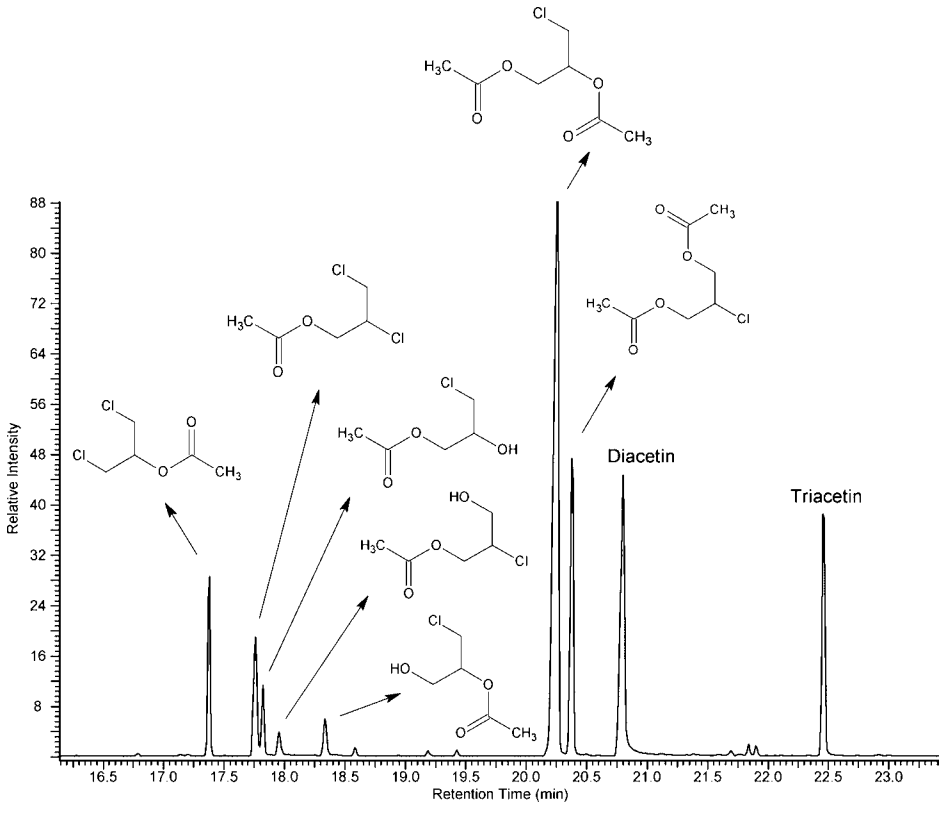

Figure 2. Generation of all possible isomers of CP acetates by the pyrolysis of pure 3-monochloropropane-1,2-diacetate at 250 °C for 20 s.

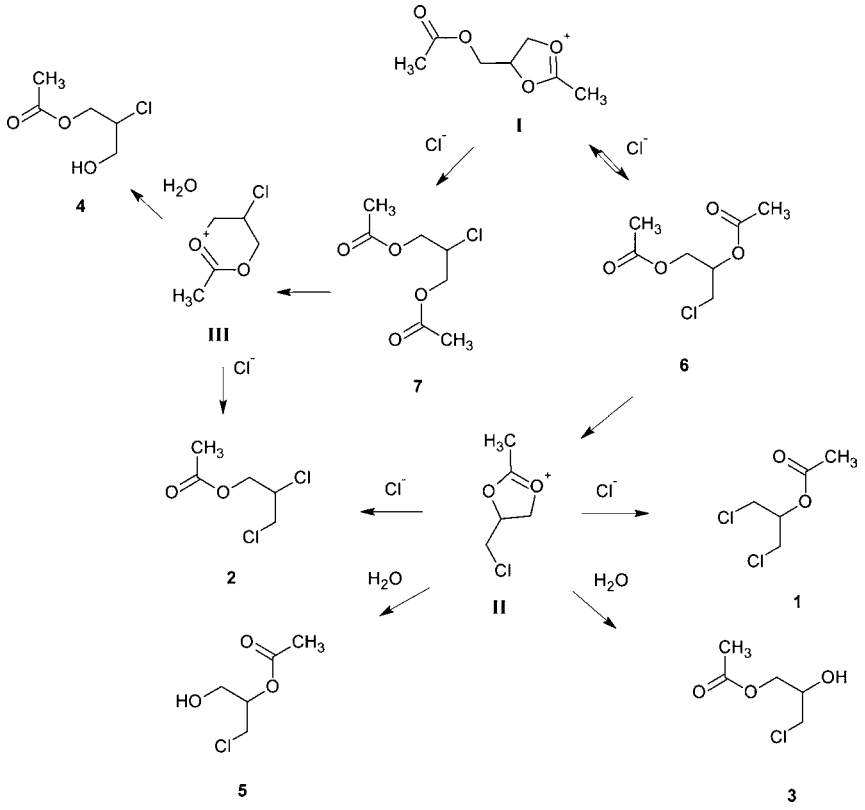

Figure 4. Proposed reaction pathway for the generation of CP acetates from 3-monochloropropane-1,2-diacetate (see also Figures 5–7).

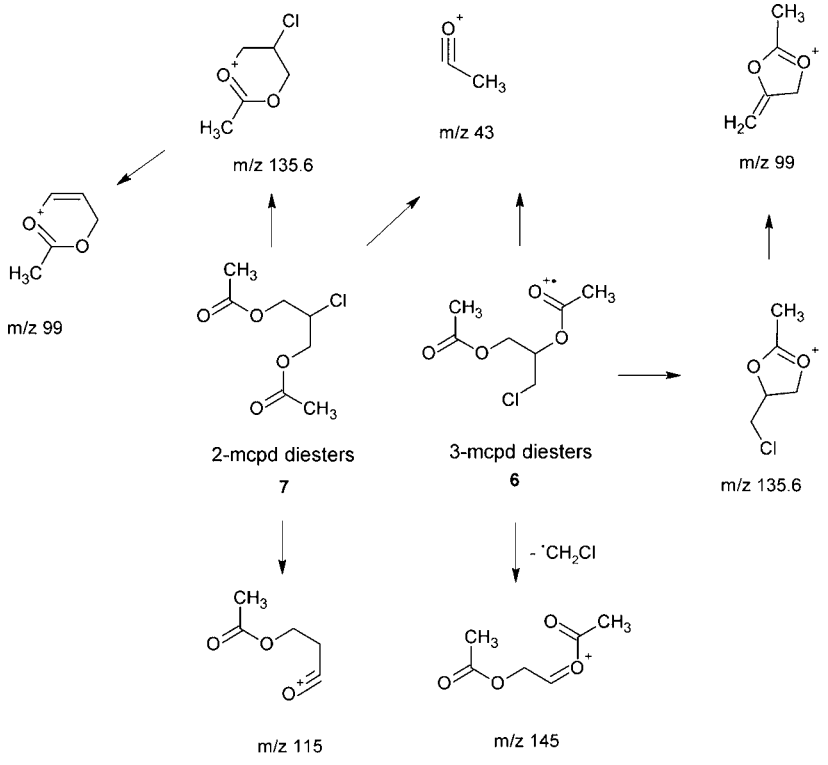

Figure 5. Proposed EI mass spectral fragmentation patterns of CP diacetates **6** and **7** (see Table 3 for the isotope incorporation data).

assessment of CP esters.[6] CP esters consisting only one type of fatty acid, such as CP acetates, shown in Figure 1, in theory can have 12 potential isomers, of which 5 are enantiomers and 7 are positional isomers. Two of seven positional isomers are 2-MCPD derivatives; three are 3-MCPD derivatives; and two are dichloro derivatives. Acknowledging the significance of differentiating between CP esters, MacMahon and colleagues[16] as well as Yamazaki and associates[17] have recently focused on developing direct methods based on LC–MS that allows for the separation of different CP esters in addition to using

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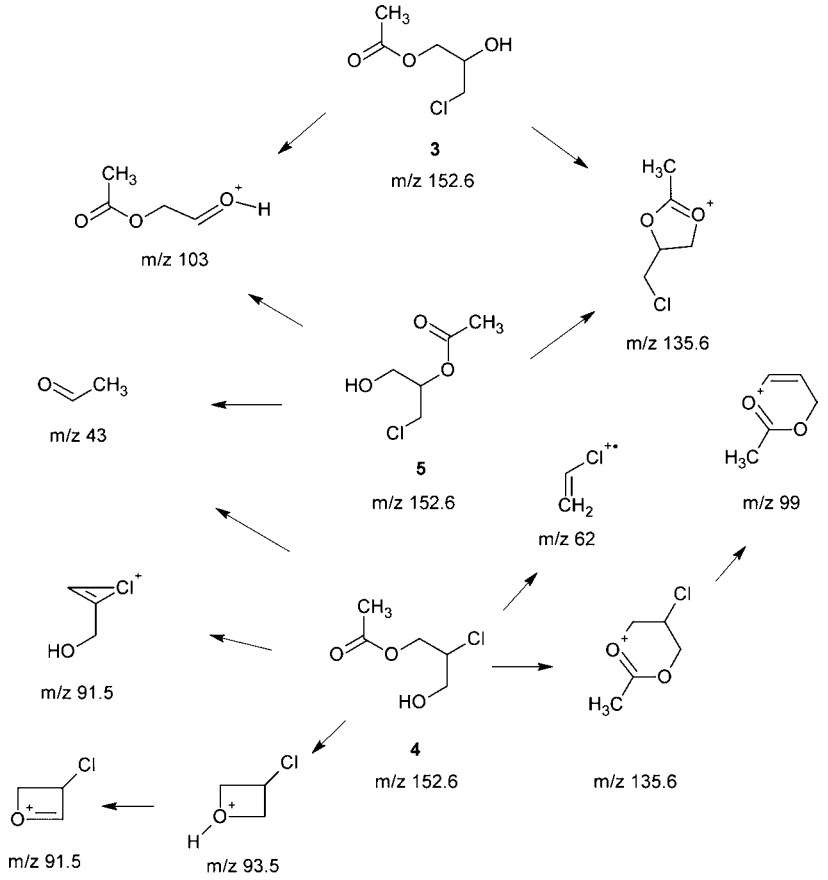

Figure 6. Proposed EI mass spectral fragmentation patterns of CP monoacetates **4** and **5** (see Table 4 for the isotope incorporation data).

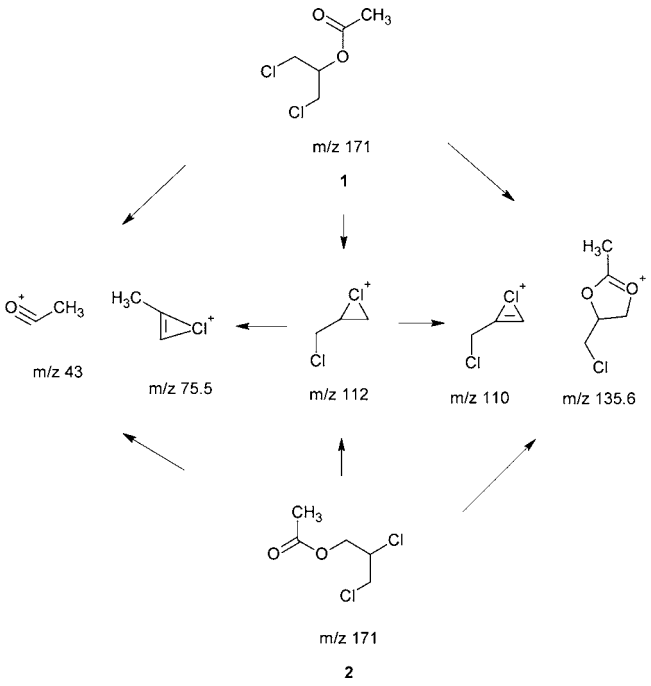

Figure 7. Proposed EI mass spectral fragmentation patterns of dichloropropanol acetates **1** and **2** (see Table 5 for the isotope incorporation data).

diagnostic fragments for their quantitation. Although both groups used the diagnostic ion $[M - RCO_2]^+$ to quantitate CP diesters, they proposed different structures for the same ion. Yamazaki's group[17] structurally represented $[M - RCO_2]^+$ as being protonated at the sn-1 position, while MacMahon and colleagues[16] referred to this ion as being cyclic in nature; this claim has been recently substantiated by Rahn and Yaylayan.[18]

Because of the lack of appropriately labeled standards to confirm fragmentation pathways of each CP ester isomer, we have developed a py–GC/MS-based method to simultaneously generate and separate all possible seven positional isomers of CP esters using acetic acid as a simple "fatty acid" model. The reaction of the various CPs with any fatty acid anhydride should generate all possible isomers, as outlined in Figure 1. According to this methodology, the co-pyrolysis of 1,3-DCP or 2,3-DCP with acetic anhydride (AA), for example, can generate information regarding the retention times and MS spectra of isomers **1** and **2** (Figure 1). Similarly, when the appropriate experiments were performed with 3-MCPD and 2-MCPD, the retention times and MS spectra of all of the remaining isomers (**3**–**7**) listed in Figure 1 could be identified on the basis of their mass differences, with the exception of isomers **3** and **5**, however, as shown in Table 2, because of almost identical mass spectra exhibited by both peaks, the assignment of their structures becomes redundant. Interestingly, when synthetic 3-MCPD diacetate (**6**) was pyrolyzed alone it generated all seven distinct isomers **1**–**7** in varying intensities, as shown in Figure 2. Similarly, when triacetin was pyrolyzed in the presence of a chloride source or when glycerol was pyrolyzed in the presence of AA and a chloride source, they also produced all seven isomers of CP acetates, as shown in Figure 3. According to the proposed pathway shown in Figure 4, these transformations can be rationalized by the initial formation of 3-MCPD diacetate

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Table 3. Isotope Incorporation Pattern and Chlorine Content of Selected Ions in the Mass Spectra of Compounds 6 and 7

A	compound **6**[a] (20.27 min)				
models	*m/z* 145	*m/z* 135	*m/z* 103	*m/z* 99	*m/z* 43
3-MCPD[b]–AA[c]	145	135	103	99	43
[^{13}C-U$_4$] AA–3-MCPD	149	137	105	101	45
[^{13}C-2,2′] AA–3-MCPD	147	136	104	100	44
[d$_5$] 3-MCPD–AA	148	140	106	103	43
[^{13}C-U$_3$] glycerol–AA[d]	147	138	105	102	43
[^{13}C-U$_4$] AA–glycerol[d]	149	137	105	101	45
[^{13}C-2,2′] AA–glycerol[d]	147	136	104	100	44
chlorine content	0	1	0	0	0
B	unknown **7**[e] (20.44 min)				
models	*m/z* 135	*m/z* 115	*m/z* 99	*m/z* 43	
2-MCPD[f]–AA	135	115	99	43	
[^{13}C-U$_4$] AA–2-MCPD	137	117	101	45	
[d$_5$] 3-MCPD–AA	140	119	103	43	
[^{13}C-U$_3$] glycerol–AA[d]	138	118	102	43	
[^{13}C-U$_4$] AA–glycerol[d]	137	117	101	45	
[^{13}C-2,2′] AA–glycerol[d]	136	116	100	44	
chlorine content	1	0	0	0	

[a]1,2-Diacetoyl-3-monochloropropane. [b]3-MCPD = 3-monochloropropane-1,2-diol. [c]AA = acetic anhydride. [d]Glycine hydrochloride was used as the chlorinating source. [e]1,3-Diacetoyl-2-monochloropropane. [f]2-MCPD = 2-monochloropropane-1,3-diol.

Table 4. Isotope Incorporation Pattern and Chlorine Content of Selected Ions in the Mass Spectra of Compounds 3–5

A	compound **5**[a] (17.75 min)					
models	*m/z* 153	*m/z* 135	*m/z* 103	*m/z* 74	*m/z* 61	*m/z* 43
3-MCPD[b]–AA[c]	153	135	103	74	61	43
[^{13}C-U$_4$] AA–3-MCPD	155	137	105	76	63	45
[^{13}C-2,2′] AA–3-MCPD	154	136	104	75	62	44
[d$_5$] 3-MCPD–AA	158	140	106	76	62	43
[^{13}C-U$_3$] glycerol–AA[d]	156	138	105	75	61	43
[^{13}C-U$_4$] AA–glycerol[d]	155	137	105	76	63	45
[^{13}C-2,2′] AA–glycerol[d]	154	136	104	75	62	44
chlorine content	1	1	0	0	0	0
B	compound **3**[e] (18.39 min)					
models	*m/z* 153	*m/z* 135	*m/z* 103	*m/z* 74	*m/z* 57	*m/z* 43
3-MCPD–AA	153	135	103	74	57	43
[^{13}C-U$_4$] AA–3-MCPD	155	137	105	76	57	45
[^{13}C-2,2′] AA–3-MCPD	154	136	104	75	57	44
[d$_5$] 3-MCPD–AA	158	140	106	76	61	43
[^{13}C-U$_3$] glycerol–AA[d]	156	138	105	75	60	43
[^{13}C-U$_4$] AA–glycerol[d]	155	137	105	76	57	45
[^{13}C-2,2′] AA–glycerol[d]	154	136	104	75	57	44
chlorine content	1	1	0	0	0	0
C	compound **4**[f] (17.97 min)						
models	*m/z* 153	*m/z* 135	*m/z* 99	*m/z* 92	*m/z* 86	*m/z* 62	*m/z* 43
2-MCPD[g]–AA	153	135	99	92	86	62	43
[^{13}C-U$_4$] AA–2-MCPD	155	137	101	92	88	62	45
[d$_5$] 3-MCPD–AA	158	140	103	96	89	65	43
[^{13}C-U$_3$] glycerol–AA[d]	156	138	102	95	88	64	43
[^{13}C-U$_4$] AA–glycerol[d]	155	137	101	92	88	62	45
[^{13}C-2,2′] AA–glycerol[d]	154	136	100	92	87	62	44
chlorine content	1	1	0	1	0	1	0

[a]2-Acetoyl-1-chloropropan-3-ol. [b]3-MCPD = 3-monochloropropane-1,2-diol. [c]AA = acetic anhydride. [d]Glycine hydrochloride was used as the chlorinating source. [e]1-Acetoyl-3-chloropropan-2-ol. [f]1-Acetoyl-2-chloropropan-3-ol. [g]2-MCPD = 2-monochloropropane-1,3-diol.

(**6**) in both model systems. As indicated above and shown in Figure 4, compound **6** is able to generate all of the remaining CP esters through the formation of the three cyclic acyloxonium ions I, II, and III[18] and their further reaction with either water or a chloride ion. The advantage of the glycerol–acetic anhydride–chloride model over the triacetin–

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Table 5. Isotope Incorporation Pattern and Chlorine Content of Selected Ions in the Mass Spectra of Compounds 1 and 2

A	compound **1**[a] (17.42 min)					
models	*m/z* 171	*m/z* 135	*m/z* 110	*m/z* 75	*m/z* 61	*m/z* 43
1,3-DCP[b]–AA[c]	171	135	110	75	61	43
[^{13}C-U$_4$] AA–1,3-DCP	173	137	110	75	63	45
[^{13}C-2,2′] AA–1,3-DCP	172	136	110	75	62	44
[d$_5$] 3-MCPD[d]–AA	176	140	114	79	63	43
[^{13}C-U$_3$] glycerol–AA	174	138	113	78	61	43
[^{13}C-U$_4$] AA–glycerol	173	137	110	75	63	45
[^{13}C-2,2′] AA–glycerol	172	136	110	75	62	44
chlorine content	2	1	2	1	0	0
B	compound **2**[e] (17.88 min)					
models	*m/z* 171	*m/z* 135	*m/z* 110	*m/z* 75	*m/z* 61	*m/z* 43
2,3-DCP[f]–AA	171	135	110	75	61	43
[^{13}C-U$_4$] AA–2,3-DCP	173	137	110	75	63	45
[^{13}C-2,2′] AA–2,3-DCP	172	136	110	75	62	44
[d$_5$] 3-MCPD–AA	176	140	114	79	63	43
[^{13}C-U$_3$] glycerol–AA	174	138	113	78	61	43
[^{13}C-U$_4$] AA–glycerol	173	137	110	75	63	45
[^{13}C-2,2′] AA–glycerol	172	136	110	75	62	44
chlorine content	2	1	2	1	0	0

[a]2-Acetoyl-1,3-dichloropropane. [b]1,3-DCP = 1,3-dichloropropanol. [c]AA = acetic anhydride. [d]3-MCPD = 3-monochloropropane-1,2-diol. [e]1-Acetoyl-2,3-dichloropropane. [f]2,3-DCP = 2,3-dichloropropanol.

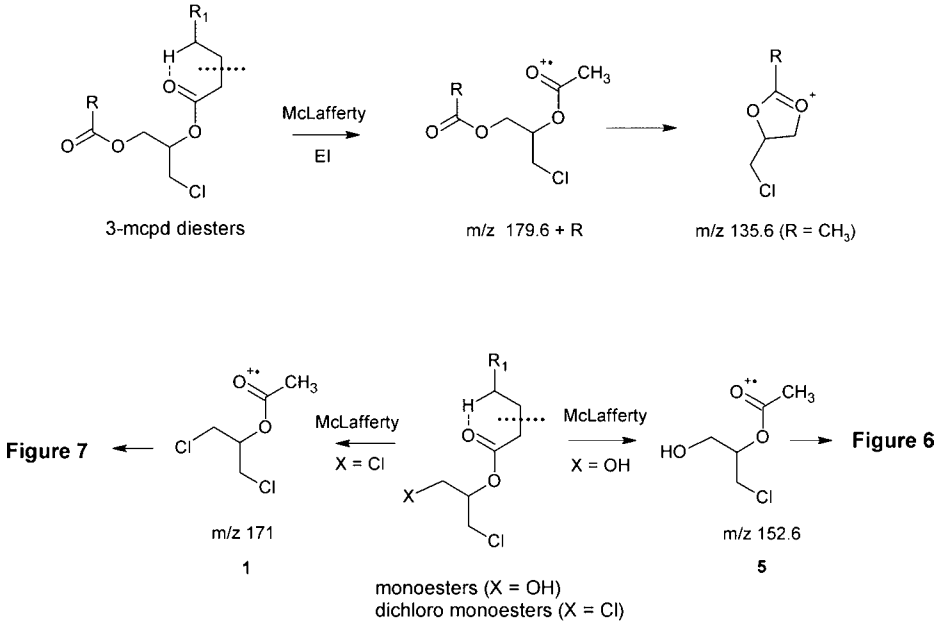

Figure 8. Conversion of long-chain CP esters subsequent to McLafferty rearrangement into CP esters containing an acetate group capable of undergoing fragmentations shown in Figures 5–7. R = fatty acid side chain, R_1 = R − C_2H_5.

chloride model is that it allows for the introduction of ^{13}C-labeled glycerol and ^{13}C-labeled acetic anhydride carbon atoms into the various CP esters through the use of [^{13}C-U$_3$] glycerol and [^{13}C-U$_4$] and [^{13}C-2,2′] acetic anhydride as reactants (see Table 1). The use of the above model systems along with a synthetic standard of 1,2-bis-acetoyl-3-chloropropane (**6**) allowed for the characterization of the EI mass spectra and the retention times of all of the positional isomers of CP acetates (Table 2).

Confirmation of EI Mass Spectral Fragmentation of CP Acetates Using the Isotope Labeling Technique. As mentioned above, reacting glycerol with acetic anhydride in the presence of a chlorine source, such as glycine hydrochloride, produced the entire array of CP acetates (see Figure 3 and Table 2). Using the natural isotope distribution pattern of chlorine as well as the systematic introduction of ^{13}C-labeled atoms from [^{13}C-U$_3$] glycerol and [^{13}C-U$_4$] or [^{13}C-2,2′] acetic anhydride into the various mass spectral fragments of CP acetates allowed us to confirm their partial elemental composition and assignment of their structures (see Figures 5–7). Two characteristic fragments were common to all of the spectra of the CP acetates, one resulting from the α cleavage of the ester moiety, leading to the formation of an intense base peak at *m/z* 43 (acylium ion), and the other an ion at *m/z* 135.6 that has been characterized previously[18] as an cyclic

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acyloxonium ion (see Figures 5−7). The acylium ion at m/z 43 dominated the spectra of all of the CP acetates studied.

The detailed fragmentation pathways of the two isomeric diesters **6** and **7** are shown in Figure 5. In both spectra, the most intense ion after the base peak was the cyclic acyloxonium ion at m/z 135.6 formed by the loss of an acyl radical. As expected, the isotope labeling studies (see Table 3) indicated the incorporation of one chlorine atom, three carbon atoms from glycerol, and C-1 and C-2 carbon atoms from acetic anhydride. The cyclic nature of the acyloxonium ion generated under EI conditions has been recently confirmed.[18] This ion subsequently undergoes dehydrohalogenation to form a weaker ion at m/z 99. In addition, the isomer **6** can also generate a characteristic ion at m/z 145 by the loss of the chloromethyl radical, and isomer **7** can generate a characteristic ion at m/z 115, albeit in low intensities. In the case of isomer **7**, a loss of a chloromethyl radical is not possible. The above ions at m/z 99, 145, and 115 exhibited isotope substitution patterns consistent with the proposed structures (Table 3).

The fragmentation pathways of the three isomeric monoesters **3**−**5** are shown in Figure 6. In all of the spectra, the most intense ion after the base peak was the cyclic acyloxonium ion at m/z 135.6; the relatively weak intensity of this ion may be due to the unfavorable requirement to lose hydroxyl free radical rather than an acyl radical for its formation. Isomer **4** was observed to exhibit a relatively intense and characteristic ion at m/z 62 containing one chlorine atom as well as two carbon atoms originating from the glycerol backbone (see Table 4), confirming the structure as an ethylene chloride radical cation. Another characteristic peak arising from isomer **4** was observed at m/z 91.5 postulated to form through acetyl radical loss. The isomers **3** and **5** on the other hand exhibited the characteristic ion at m/z 103 generated by the loss of the chloromethyl radical based on the isotope labeling data (Table 4). Unlike isomers **6** and **7**, the three isomeric monoesters also showed molecular ions at m/z 153, with an isotopic labeling pattern consistent with singly chlorinated structures. Isotope labeling studies (see Table 4) were consistent with the proposed fragments shown in Figure 6.

In general, the dichlorinated isomers of CP esters have been reported to be less abundant in foods,[20] with the 1,3-dichloro isomer being the most abundant. The mass spectra obtained of the dichlorinated acetates **1** and **2** revealed virtually identical fragmentations occurring upon ionization, as shown in Figure 7. Similar to isomers **3**−**5**, dichlorinated isomers also showed weak ions at m/z 135.6, again indicating the acyl radicals as preferred leaving groups relative to chlorine or hydroxyl radicals in undergoing displacement rearrangement to generate cyclic acyloxonium ions. Consistent with the above observation, a similar displacement rearrangement reaction initiated by the chlorine radical cation can displace the acyl radical to form this time chloronium ion at m/z 112, which can undergo further transformations to generate ions at m/z 75.5 and 110. Isotope labeling studies (see Table 5) were consistent with the proposed structures shown in Figure 7.

Advantages of Using CP Acetates as Model CP Esters. In addition to the ease of generation of all possible members of the CP acetates and their isotopically labeled counterparts, the main advantage of using acetate esters is the fact that they also represent an important fatty acid moiety that forms from longer chain fatty acids (R) after McLafferty rearrangement,[21−23] as shown in Figure 8. For example, any 3-MCPD diester after undergoing McLafferty rearrangement can generate an acetate moiety at m/z (179.6 + R), which subsequently can generate the ion at m/z 135.6, as shown in Figure 8. On the other hand, 3-MCPD monoesters that undergo McLafferty rearrangement can generate compounds **1** and **5** depending upon the nature of the substituent at the sn-1 position of the glycerol backbone, as shown in Figure 8. According to Figure 7, compound **1** will generate characteristic ions, such as ions at m/z 171, 112, 110, and 76, and compound **5** can generate characteristic ions at m/z 152.6 and 103, as described in Figure 6.

In general, the nature of the mass spectral fragments of CP diesters will primarily depend upon the fatty acid chain length, such as ions at m/z (28 + R), (120.6 + R), and (179.6 + R) shown in Figure 8, with the exception of the ion at m/z 135.6. This chlorinated cyclic oxonium ion is expected to be formed in all CP esters subsequent to McLafferty rearrangement and, as such, can be considered as a universal marker for the presence of CP esters. Its relative abundance is expected to vary depending upon the ease of its formation, which, in turn, depends upon the nature of the neighboring group that is being displaced. The highest intensities of this ion were encountered when the neighboring group was an ester followed by an alcohol and then a chlorine atom. The loss of a chlorine atom and the formation of the corresponding cyclic acyloxonium ion at m/z 159 were not observed in the mass spectra of CP diacetates studied; nevertheless, MacMahon and colleagues[16] reported successful use of this ion for quantitation of 3-MCPD diesters.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Hamlet, C. G.; Sadd, P. A. Chloropropanols and chloroesters. In *Process-Induced Food Toxicants: Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R. H., Lineback, D. R., Eds.; John Wiley and Sons: Hoboken, NJ, 2009; pp 175−214.

(2) Rahn, A. K. K.; Yaylayan, V. A. What do we know about the molecular mechanism of 3-MCPD ester formation? *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 323−329.

(3) Velíšek, J. Chloropropanols. In *Process-Induced Food Toxicants: Occurrence, Formation, Mitigation, and Health Risks*; Stadler, R. H., Lineback, D. R., Eds.; John Wiley and Sons: Hoboken, NJ, 2009; pp 539−562.

(4) Nagy, K.; Sandoz, L.; Craft, B. D.; Destaillats, F. Mass-defect filtering of isotope signatures to reveal the source of chlorinated palm oil contaminants. *Food Addit. Contam., Part A* **2011**, *28*, 1492−1500.

(5) Crews, C.; Chiodini, A.; Granvogl, M.; Hamlet, C.; Hrnčiřík, K.; Kuhlmann, J.; Lampen, A.; Scholz, G.; Weisshaar, R.; Wenzl, T.; Jasti, P. R.; Seefelder, W. Analytical approaches for MCPD esters and glycidyl esters in food and biological samples: A review and future perspectives. *Food Addit. Contam., Part A* **2013**, *30*, 11−45.

(6) Seefelder, W.; Scholz, G.; Schilter, B. Structural diversity of dietary fatty esters of chloropropanols and related substances. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 319−322.

(7) Hamlet, C. G.; Asuncion, L. Single-laboratory validation of a method to quantify bound 2-chloropropane-1,3-diol and 3-chloropropane-1,2-diol in foodstuffs using acid catalysed transesterification, HFBI derivatisation and GC/MS detection. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 345−355.

(8) Hrncirik, K.; Zelinková, Z.; Ermacora, A. Critical factors of indirect determination of 3-chloropropane-1,2-diol esters. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 361−367.
(9) FEDIOL. *FEDIOL Recommendations on the Methodology To Be Used for the Analysis of 3-MCPD Esters and Glycidol Esters in Oils and Fats*; FEDIOL: Brussels, Belgium, 2010; 10SAF082Final, http://www.fediol.eu/data/10SAF082Final_FEDIOL_recommendations_on_analytical_methods_3MCPD_esters_glycidol_esters_24April.pdf (accessed March 1, 2013).
(10) Collison, M. W. Direct determination of MCPD esters and glycidol esters by LCMS. *OVID Meeting*; Berlin, Germany, Jan 25, 2010; http://www.ovid-verband.de/fileadmin/user_upload/ovid-verband.de/downloads/ADM_Collison.pdf (accessed March 1, 2013).
(11) Gardner, A. M.; Yurawecz, M. P.; Cunningham, W. C.; Diachenko, G. W.; Mazzola, E. P.; Brumley, W. C. Isolation and identification of C16 and C18 fatty acid esters of chloropropanediol in adulterated Spanish cooking oils. *Bull. Environ. Contam. Toxicol.* **1983**, *31*, 625−630.
(12) Davídek, J.; Velíšek, J.; Kubelka, V.; Janíček, G.; Šimicová, Z. Glycerol chlorohydrins and their esters as products of the hydrolysis of tripalmitin, tristearin and triolein with hydrochloric acid. *Z. Lebensm.-Unters. Forsch.* **1980**, *171*, 14−17.
(13) Velíšek, J.; Davídek, J.; Kubelka, V.; Bartošová, J.; Tučková, A.; Hajšlová, J.; Janíček, G. Formation of volatile chlorohydrins from glycerol (triacetin, tributyrin) and hydrochloric acid. *Lebensm.-Wiss. Technol.* **1979**, *12*, 234−236.
(14) Kraft, R.; Brachwitz, H.; Etzold, G.; Langen, P.; Zöpfl, H.-J.; Halogenlipide, I. Massenspektrometrische strukturuntersuchung stellungsisomer fettsäureester der halogenpropandiole (desoxyhalogenglyceride). *J. Prakt. Chem.* **1979**, *321*, 756−768.
(15) Brachwitz, H.; Kraft, R.; Langen, P.; Etzold, G.; Schildt, J. Halogenlipide II: Zur synthese und massenspektrometrischen charakterisierung von 2-*O*-acyl-3-halogen-propan-1,2-diolen. *J. Prakt. Chem.* **1979**, *321*, 769−774.
(16) MacMahon, S.; Begley, T. H.; Diachenko, G. W. Analysis of processing contaminants in edible oils. Part 2. A liquid chromatography tandem mass spectrometry method for the direct detection of 3-monochloropropanediol (3-MCPD) and 2-monochloropropanediol (2-MCPD) di-esters. *J. Agric. Food Chem.* **2013**, *61*, 4748−4757.
(17) Yamazaki, K.; Ogiso, M.; Isagawa, S.; Urushiyama, T.; Ukena, T.; Kibune, N. A new, direct analytical method using LC−MS/MS for fatty acid esters of 3-chloro-1,2-propanediol (3-MCPD esters) in edible oils. *Food Addit. Contam.* **2013**, *30*, 52−68.
(18) Rahn, A. K. K.; Yaylayan, V. A. Cyclic acyloxonium ions as diagnostic aids in the characterization of chloropropanol esters under EI, ESI and APCI conditions. *J. Agric. Food Chem.* **2013**, *61*, 5946−5952.
(19) Lebensmittelchemisches Institut des Bundesverbandes der Deutschen Süßwarenindustrie (LCI). *Wieviele 3-MCPD-Ester gibt es—Ein Rechenmodell*; LCI: Cologne, Germany, 2008; http://www.lci-koeln.de/deutsch/veroeffentlichungen/lci-focus/wieviele-3-mcpd-ester-gibt-es-ein-rechenmodell/ (accessed March 1, 2013).
(20) Šmidrkal, J.; Ilko, V.; Filip, V.; Doležal, M.; Zelinková, Z.; Kyselka, J.; Hrádková, I.; Velíšek, J. Formation of acylglycerol chloro derivatives in vegetable oils and mitigation strategy. *Czech J. Food Sci.* **2011**, *29*, 449−456.
(21) Aasen, A. J.; Lauer, W. M.; Holman, R. T. Mass spectrometry of triglycerides. II. Specifically deuterated triglycerides and elucidation of fragmentation mechanisms. *Lipids* **1970**, *5*, 869−877.
(22) Lauer, W. M.; Aasen, A. J.; Graff, G.; Holman, R. T. Mass spectrometry of triglycerides: I. Structural effects. *Lipids* **1970**, *5*, 861−868.
(23) Morrison, A.; Barratt, M. D.; Aneja, R. Mass spectrometry of some deuterated 1,3-distearins. *Chem. Phys. Lipids* **1970**, *4*, 47−59.