JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Cyclic Acyloxonium Ions as Diagnostic Aids in the Characterization of Chloropropanol Esters under Electron Impact (EI), Electrospray Ionization (ESI), and Atmospheric Pressure Chemical Ionization (APCI) Conditions

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ABSTRACT: During mass spectrometric analysis of various lipids and lipid derivatives such as the chlorinated counterparts of triacylglycerols, the detailed structure of the characteristic and common ions formed under electron impact (EI), electrospray ionization (ESI), and atmospheric pressure chemical ionization (APCI) conditions by the loss of a single fatty acid remains ambiguous. These ions are designated in the literature as "diacylglyceride ions" and are frequently depicted with a molecular formula without showing any structural features and sometimes represented as cyclic acyloxonium ions. Characterization of these ions is of considerable importance due to their utility in structural identification of lipid derivatives. This study provides complementary evidence on the cyclic nature of "diacylglyceride ions" through the use of the simplest 3-monochloropropanediol diester as a model and the use of isotope labeling technique. Tandem MS/MS studies have indicated that the ion at m/z 135.6 generated from 1,2-bis(acetoyl)-3-chloropropane through the loss of an acetyl group was identical to the ion at m/z 135.6 generated from 4-chloromethyl-2,2-dimethyl-1,3-dioxolane, the latter being generated from a cyclic precursor through the loss of a methyl radical, keeping the dioxolane ring structure intact, thus confirming the cyclic nature of these ions. The corresponding cyclic oxonium ions generated from longer chain chloropropanol diesters, such as the ion at m/z 331.2 originating from 3-monochloropropanediol (3-MCPD) diesters containing palmitic acid(s), could serve as chemical markers for the presence chloropropanol esters.

KEYWORDS: *chloropropanol diesters, lipids, cyclic acyloxonium ions, diagnostic ions, isotope labeling*

INTRODUCTION

Under various soft ionization conditions, protonated triacylglycerols (TAGs) generate characteristic mass spectral fragments $[M - RCO_2]^+$ resulting from the loss of a single fatty acid.[1,2] Generally referred to as diacylglyceride ions,[3] these fragments are often designated in the literature as $[AA]^+$ ions, in the case of TAGs containing three identical fatty acids (AAA), which in turn can be structurally represented by **1** or **1′** shown in Figure 1. Similar fragments are generated under electron impact conditions through a displacement rearrangement (rd) mechanism, originating from the parent radical cation as shown in Figure 1. Such ions have been used extensively for the quantitative and qualitative analysis of lipids.[4] Mixed TAGs of the type ABA or ABC can generate up to three such diacylglyceride ions ($[AB]^+$, $[AC]^+$, $[AA]^+$) in various intensities (where A, B, and C represent different fatty acids). The least abundant among these ions originates through the loss of fatty acids located at the sn-2 position.[3−5] This fact can be used to differentiate between two regioisomers such as ABA and AAB, on the basis of the variance in the abundance of the corresponding $[M - RCO_2]^+$ fragments such as $[AA]^+$ and $[AB]^+$ produced.[5,6] Although the abundances of these fragments can also vary as a function of the fatty acid chain length or their molecular weights, appropriate calibration can overcome these limitations.[7] In addition to TAGs, diacylglycerols[8] and chloropropanol (CP) esters also exhibit prominent mass spectral peaks corresponding to the loss of a fatty acid moiety as shown in Figure 1, where the functional group being lost is regulated this time by the leaving group hierarchy (halides > hydronium ions > esters > alcohols),[9] and are influenced by the mode of ionization applied. Protonation occurring during ionization will convert the free hydroxyl group into alkyl oxonium ion,[1,8,10] which may undergo preferential cleavage over the ester groups.

Although chloropropanols were initially identified in hydrolyzed vegetable proteins (HVP) during the late 1970s,[11] they have recently become more prominent[12] due to the discovery of their esters in refined edible oils such as palm oil. Consequently, a closer look at their mass spectral fragmentation pattern is required; especially the structure of their most prominent mass spectral peaks remains ambiguous. These ions are frequently depicted in the literature with a molecular formula without showing any structural features,[12,13] and only a few studies have attempted to assign specific chemical structures such as cyclic acyloxonium ions to these mass spectral fragments.[14,15] In Figure 1, such ions are shown as equilibrium structures between cyclic acyloxonium ion (**1**) and a carbocation (**1′**). Distinguishing between the two forms can have significant implications in the predictive value of these

Received: March 12, 2013
Revised: May 27, 2013
Accepted: June 4, 2013
Published: June 4, 2013

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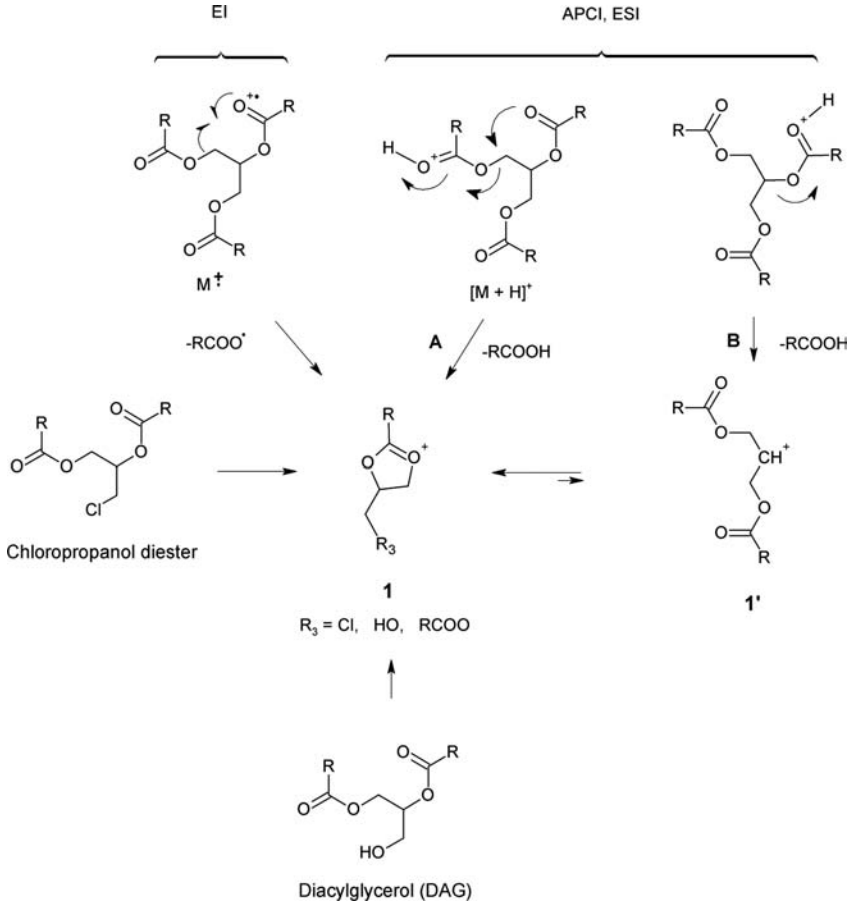


Figure 1. Loss of fatty acid moieties from triacylglycerols, diacylglycerols, and chloropropanol diesters under electron impact (EI), electrospray ionization (ESI), and atmospheric pressure chemical ionization (APCI) conditions to generate cyclic acyloxonium ion (**1**) or its carbocation counterpart (**1′**).

ions. In this study we provide evidence in support of the cyclic nature of these ions.

MATERIALS AND METHODS

Materials. Triacetin (99+%), glycine hydrochloride (99%), 3-chloro-1,2-propanediol (98%), 2,3-dichloro-1-propanol (>97.0%), glycerol anhydrous (>99.5%), 1,2-dipalmitoyl-*rac*-glycerol (99%), 1-palmitoyl-glycerol (99%), and 4-chloro-2,2-dimethyl-1,4-dioxolane (98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-Chloro-1,2-propanediol (propane-d_5, 98%), acetic anhydride (1,1′, 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, USA); 1,3-dichloro-2-propanol (98%) was purchased from ACP Chemicals Inc. (Montreal, Quebec, Canada); acetic anhydride (≥97%) was purchased from Anachemia, Lachine, Quebec, Canada. 2-Chloro-1,3-propanediol (98%) and *rac*-1,2-bis(palmitoyl)-3-chloropropanediol (98%) were purchased from Toronto Research Chemicals (Ontario, Canada). All chemicals were used without further purification. The ^{13}C and ^{1}H NMR spectra were acquired in $CDCl_3$ on a 400 MHz Varian Unity spectrometer (Varian Associates, Palo Alto, CA, USA). Infrared spectra were recorded on a Bruker Alpha-P spectrometer (Bruker OpticGmbH, Ettlingen, Germany) equipped with a deuterated triglycine sulfate (DTGS) detector, a temperature-controlled single-bounce diamond attenuated total reflectance (ATR) crystal, and a pressure application device for solid samples. Processing of the FTIR data was performed using Bruker OPUS software (Bruker Optic GmbH).

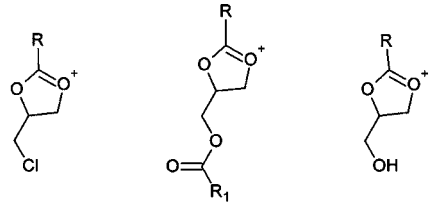
Pyrolysis–Gas Chromatography–Mass Spectrometry (Py-GC-MS) Analyses. A Varian CP-3800 gas chromatograph coupled to a Saturn 2000 ion trap detector interfaced to a CDS Pyroprobe 2000 unit through a valved interface (CDS 1500) was used for Py-GC-MS analysis. In all experiments (see Tables 1 and 2), the triacetin/glycine hydrochloride model mixtures (1 mg, 1:2 molar ratio) were introduced inside the quartz tube (0.3 mm thickness), which was plugged with quartz wool, and inserted into the coil probe. The temperature of the

Table 1. Selected Examples from the Literature on the Formation of Diglyeride Ions in TAGs and Chloropropanol Diesters

compound	diglyceride ion (m/z)
tripalmitin	551[a,b]
dipalmitin	551[c], 313[c]
monopalmitin	313[d]
palmitoyl-MCPD monoester	313.2[e,f]
oleoyl-MCPD monoester	339.2[e,f]
dipalmitoyl-MCPD	331.2[e,g,h]
palmitoyl-oleoyl-MCPD	331.2[e,g]
palmitoyl-oleoyl-MCPD	357.2[e,g]
dioleoyl-MCPD	357.2[b,g,f,h]
distearoyl-MCPD	360.0/359[g,f,h]
stearoyl-MCPD	341.5[f]
1-palmitoyl-2-oleoyl-MCPD	357[i]
stearoyl-oleoyl-MCPD	359[g], 357 [g]
stearoyl-palmitoyl-MCPD	359[g], 331 [g]
APCI/ESI references	[g]20; [e]12; [i]19; [a]2; [c] 28
EI references	[h]26; [f]22; [b]27

dx.doi.org/10.1021/jf401118n | J. Agric. Food Chem. 2013, 61, 5946–5952

Table 2. Cyclic Acyloxonium Ions Observed from Selected TAGs and CP Esters under ESI and APCI Conditions Using Sodiation and Protonation



Precursors	Acyloxonium Ion I		Acyloxonium Ion II		Acyloxonium Ion III	
	APCI	ESI	APCI	ESI	APCI	ESI
Monopalmitin	-	-	-	-	313.11[a]	**313.5**[b]
Dipalmitin	-	-	551.29[a]	551.6[b]	313.14[a]	**313.5**[b]
Tripalmitin	-	-	**551.31**[a]	**551.6**[b,a]	-	-
1,2-Diacetyl-3-monochloropropane	**135.02**[a]	135.02[a]	158.97[a]	**158.96**[a]	-	-
1-Myristoyl-3-monochloropropan-2-ol	303.11[a]	-	-	-	285.04[a]	-
2-Palmitoyl-3-monochloropropan-1-ol	331.12[a]	-	-	-	**313.09**[a]	313.22[a]
1,2-Dipalmitoyl-3-monochloropropane	331.27[a]	331.4[b]	**551.60**[a]	**551.20**[a]	-	-
1-Palmitoyl-2-stearoyl-3-	**359.19**[a]	-	579.36[a]	579.28[a]	-	-
monochloropropane	331.17[a]	-	-	-	-	-

[a] under sodiation; [b] under protonation

Table 3. Model Systems Used and Percent Label Incorporation in the Ion at *m/z* 135 Originating from 1,2-Bis(acetoyl)-3-chloropropane

	% label incorporation[a]					
model system	M	M + 1	M + 2	M + 3	M + 4	M + 5
triacetin + Gly.HCl[b]	100	0	0	0	0	0
glycerol + AA[c] **+ Gly.HCl**[b]	100	0	0	0	0	0
glycerol + [^{13}C-2,2′]AA + Gly.HCl[b]	0	100	0	0	0	0
glycerol + [^{13}C-U$_4$]AA + Gly.HCl[b]	0	0	100	0	0	0
[^{13}C-U$_3$]glycerol + AA + Gly.HCl[b]	0	0	0	100	0	0
3-MCPD[d] **+ AA**	100	0	0	0	0	0
3-MCPD + [^{13}C-2,2′]AA	0	100	0	0	0	0
3-MCPD + [^{13}C-U$_4$]AA	0	0	100	0	0	0
[d$_5$]3-MCPD + AA	0	0	0	0	0	100

[a] All ions also showed incorporation of one chlorine atom. [b] Glycine hydrochloride used as a source of HCl. [c] AA, acetic anhydride. [d] 3-MCPD, 3-monochloropropanediol.

pyroprobe interface was set at 250 °C. Model systems were initially pyrolyzed at 250 °C with a total heating time of 20 s. Samples were left in the interface for a total of 2 min and then removed. After 5 min, the sample was introduced into the interface 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 capillary direct MS interface temperature was 250 °C; 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 i.d., and 0.33 μm film thickness; J&W Scientific). The identity and purity of the chromatographic peaks were determined by using NIST AMDIS version 2.1.

In Situ Pyrolytic Generation of Labeled 1,2-Bis(acetoyl)-3-chloropropanediols. In the above experiments the triacetin/glycine hydrochloride model mixtures were replaced with either 3-chloro-1,2-propanediol/acetic anhydride (1,1′, 2,2′-^{13}C$_4$) or glycerol (^{13}C$_3$)/acetic anhydride/glycine hydrochloride (1 mg, 1:2 molar ratio) mixtures (see also Table 3) or their unlabeled counterparts and pyrolyzed as indicated above. The formation of the title compound was confirmed through comparison of their retention times and mass spectra to those of a synthetic sample of 1,2-bis(acetoyl)-3-chloropropanediol (see below).

Py-GC-MS/MS Analysis. Using the identical equipment setup as described above for Py-GC-MS, samples (1.0 mg) of either 3-monochloropropane-1,2-diol diacetate or 4-chloro-2,2-dimethyl-1,4-dioxolane diluted 10 times (w/w) with silica were introduced inside the quartz tube (0.3 mm thickness), which was plugged with quartz wool, and inserted into the coil probe. An identical GC column as well as the same temperature−pressure methodology was used in the analysis. After the initial fragmentation was carried out, the ion at *m/z* 135 was isolated with a window of *m/z* 3.0 and fragmented further using resonant waveform and an excitation storage level of *m/z* 59.3.

dx.doi.org/10.1021/jf401118n | J. Agric. Food Chem. 2013, 61, 5946−5952

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis. Two milligram samples of chloropropanol ester standards were diluted in an autosampler vial (1 mL) with 0.5 mL of the same 1:4 (v/v) 2-propanol/hexane mixture. A Hewlett-Packard GC with a mass selective detector (5890 GC/5971B MSD) interfaced to a CDS pyroprobe 2000 unit was used for the Py-GC-MS analysis. One microliter samples of reactants were injected using a Henderson syringe into the pyrolysis interface, which was flushed with helium at a rate of 20 mL/min for the first 2 min. Separation was carried out on an HP1 (100% dimethylpolysiloxane) capillary column having a 12.5 m length, a 0.22 mm internal diameter, and a 0.33 μm film thickness (Hewett-Packard), using helium as the carrier gas. The initial temperature of the column was set at 45 °C for 3 min before being raised to 150 °C at a rate of 10 °C/min. The temperature was then immediately raised to 300 °C, at a rate of 20 °C/min, and held there for 5 min before being raised to 350 °C again at a rate of 20 °C/min, which was held for another 5 min. A constant flow of 1 mL/min was held throughout separation. Compounds separated were detected using a quadrupole mass spectrometer using an ionization voltage of 70 eV and an EMV of 1400 V, along with a scanning range of m/z 25–650. The MS transfer line was set at 250 °C. The identity and purity of the chromatographic peaks were determined by using NIST AMDIS version 2.1.

ESI-MS Analysis of Lipid Samples Using Sciex API 4000. The ESI-MS spectra were obtained using a Sciex API 4000 triple-quadrupole mass spectrometer (Sciex, Concord, Canada). Samples (see Table 2 samples designated with superscript *b*) were analyzed in the positive ionization mode using the following parameters: the turbo ion spray source was at 450 °C with ion spray voltage at 5500 V, curtain gas (N_2) at 10 psi, ion source nebulizer gas at 61 psi, and ion source turbo gas at 36 psi. During the experiment the entrance potential was set at 10 V and the declustering potential was set at 100 V. Samples were infused into the electrospray ion source through a Cole Parmer Infusion pump model 74900 with a 1 mL syringe at a rate of 2 mL/h. The scan range was between 150 and 2500 amu.

ESI-MS Analysis of Lipid Samples Using a Thermo Finnigan LCQ. The ESI-MS spectra were obtained using a Thermo Finnigan LCQ quadrupole mass spectrometer (San Jose, CA, USA). Samples (see Table 2 samples designated with superscript *a*) were analyzed in the positive ionization mode while being infused at a rate of 5 μL/min into an unheated ESI interface. The needle voltage was 4.5 kV using nitrogen as the curtain gas and a scan range between 150 and 1000 amu.

APCI-MS Analysis of Lipid Samples. The APCI-MS spectra were obtained using a Thermo Finnigan LCQ quadrupole mass spectrometer. Samples (see Table 2) were analyzed in the positive ionization mode and infused at a rate of 5 μL/min into the APCI interface and were evaporated at 450 °C. The needle voltage was 4.5 kV using nitrogen as the curtain gas and a scan range between 150 and 1000 amu.

Synthesis of *rac*-1,2-Bis(acetoyl)-3-chloropropanediol. A mixture of 3-monochloropropanediol (3-MCPD) (0.26 g) and excess acetic anhydride (1.3 g) was heated on a sand bath at 120 °C for 15 min in an open vial followed by the addition of a catalytic amount of methanolic HCl with continued heating for 1.5 h to evaporate excess anhydride, yielding the title compound as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$), δ 5.10(m, 1H, H-3), 4.29–4.09 (m, 2H, H-4, 5), 3.62–3.53 (m, 2H, H-1, 2), 2.00 (s, 3H, H-1′, 2′, 3′), 1.98 (s, 3H, H-1″, 2″, 3″); ^{13}C NMR, δ 170.26 and 169.83 (C-6, 4), 70.35 (C-2), 62.29 (C-3), 42.08 (C-1), 20.66 and 20.52 (C-7, 5); ATR-FTIR (oil), 3040–2960 cm^{-1} (alkyl stretch), 2960–2860 cm^{-1} (C—H stretch), 1738 cm^{-1} (C═O stretch), 1300–1150 cm^{-1} (C—O—C stretch), 753 cm^{-1} (C—Cl stretch); MS, m/z (% abundance) 39 (3.0), 42 (5.2), 43 (100), 44 (3.7), 88 (1.9), 99 (8.0), 101 (2.2), 103 (12.9), 135 (6.4), 137 (2.1).

RESULTS AND DISCUSSION

Chloropropanol esters (CP esters) and acylglycerols are commonly analyzed by liquid chromatography (LC) coupled to a soft ionization technique such as electrospray (ESI) or atmospheric pressure chemical (APCI) ionization (see Table 1). Ionization is usually achieved through the addition of formic acid, ammonia, sodium, or lithium salts to the mobile phase, forming adducts between the reagent and the analyte(s) ($[M + X]^+$) that are responsible for the generation of characteristic mass spectra. The fragments generated upon collision-induced dissociation (CID) are characteristic of this adduct and may not contain the ionizing reagent; these fragments are considered to be intrinsically cationic in nature such as ion **1** depicted in Figure 1.[4,5,16] Not all reagents (ammonia vs lithium, for example, in the analysis of TAGs containing unsaturated fatty acids), however, will generate the same set of fragments; some reagents, for example, will not associate at the same site within the molecule, influencing their subsequent fragmentation pathway and thus preventing these ions from being considered as universal diagnostic ions.[7,16] No attempt has been made in the literature to confirm whether ions such as **1** are indeed unprotonated and cationic in nature.

Confirmation of Intrinsic Cationic Nature of the Characteristic Mass Spectral Fragments from TAGs and CP Diesters under ESI and APCI Conditions. To provide further evidence that these ions are indeed unprotonated, the abilities of different ionization conditions (sodiation vs protonation) to generate the cationic species resulting from the loss of a single fatty acid from a group of TAGs and CP esters were studied under comparable conditions (see Table 2). In the absence of high-resolution data, sodiation can be considered the only reliable ionization technique that can irrefutably generate unprotonated cationic species because the use of ammonia $[M + NH_4]^+$ can also generate $[M + H]^+$ upon degradation.[7] In addition, the advantage of using sodium is that it behaves in a similar manner to protonating and ammoniating reagents but the resulting adduct demonstrates a greater mass difference, relative to a proton loss, generating a more pronounced mass signature if sodium was present in the CID fragments.[17] Thus, the absence of sodium in a positively charged mass fragment can be considered as a confirmation of intrinsically cationic species. Mass spectral analysis of selected acylglycerols, CP mono- and diesters under sodiation and protonation conditions, generated identical ions as listed in Table 2, indicating the formation of unprotonated cationic species I, II, and III. The cyclic nature of these ions can be indirectly inferred from a recent study[18] indicating that terminal fatty acids in the sn-1 or sn-3 position in the glycerol backbone of TAGs are more likely to be cleaved, that is, energetically favored, than a fatty acid at the sn-2 position.[3,6,16] This regiospecific bias can be seen by the ion intensities where the fragments resulting from the cleavage of sn-2 fatty acids are weak relative to the intensities of ions generated from fatty acid cleavage from sn-1 and sn-3.[18] These observations are consistent with pathway A rather than pathway B illustrated in Figure 1. Pathway A can be considered as an intramolecular S_N2 type reaction requiring the loss of fatty acid from less hindered carbon atom with simultaneous formation of ion **1** and pathway B as S_N1 type reaction requiring the formation of the most stable carbocation by the loss of fatty acid from a secondary carbon atom and formation of ion **1′**. Therefore, a preferential loss of FA from sn-1/-3 suggests a mechanism sensitive to steric hindrance (intramolecular S_N2 mechanism) forming a cyclic structure **1** rather than the formation of the most stable carbocation **1′** at position sn-2. The cyclic nature is further supported by Marzilli and associates.[6] Theorizing that

dx.doi.org/10.1021/jf401118n | J. Agric. Food Chem. 2013, 61, 5946–5952

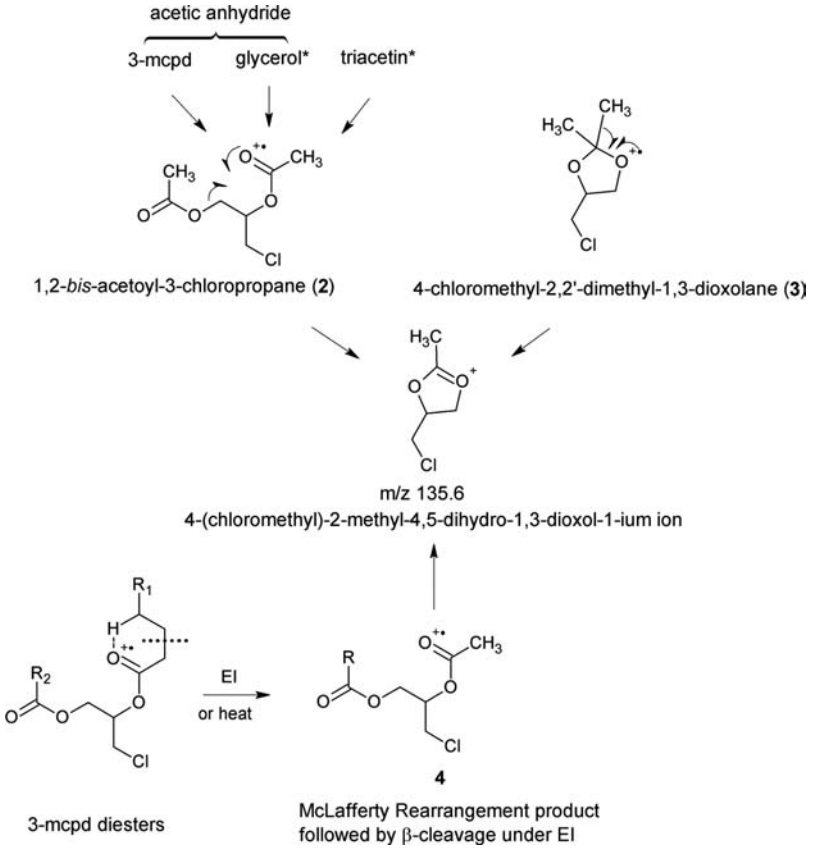


Figure 2. Generation of the common ion at m/z 135.6 from 1,2-bis(acetoyl)-3-chloropropane, 4-chloromethyl-2,2′-dimethyl-1,3-dioxolane and from McLafferty rearrangement product of any 3-MCPD diester under electron impact (EI) conditions. * indicates the reaction was performed in the presence of a chloride source such as glycine.HCl.

$[M - RCO_2]^+$ was cyclic in nature, Marzilli and colleagues[8] conducted tandem MS experiments on identical $[M - RCO_2]^+$ fragments originating from a set of regioisomers such as 1,2-distearoyl-3-oleoylglycerol and 1,3-distearoyl-2-oleoylglycerol. Obtaining identical mass spectra from the MS^n of $[M - RCO_2]^+$ between two regioisomers demonstrated to Marzilli et al.[6] that the structural identity had been lost and that intramolecular cyclization had likely occurred during their formation.

Evidence for the Formation of Cyclic Acyloxonium Species under EI Conditions. Although under soft ionization conditions relative intensities of the diacylglyceride ions can provide ample evidence on the mechanism of formation of cyclic acyloxonium ions, no such information is available on the relative intensities of these ions under EI conditions. To investigate the cyclic nature of $[M - RCO_2]^+$ ions under EI conditions the simplest CP diester, 1,2-bis(acetoyl)-3-chloropropane (**2** in Figure 2), was used as a model to allow the introduction of isotopically labeled carbon atoms into the structure of **2** through copyrolysis of labeled precursors. Copyrolysis of a mixture of acetic anhydride and glycerol in the presence of a chloride source or, alternatively, copyrolysis of a mixture of 3-MCPD and acetic anhydride can generate 1,2-bis(acetoyl)-3-chloropropane (**2**) as shown in Figure 2. The formation was verified through independent synthesis of **2** and comparison of their retention times as well as mass spectra. The two in situ synthesis methods mentioned above allowed the introduction of either $[^{13}C\text{-}U_3]$glycerol backbone into the structure **2** or ^{13}C-labeled acetyl groups through the use of either $[^{13}C\text{-}U_4]$- or $[^{13}C\text{-}2,2']$acetic anhydride (see Table 3). Furthermore, copyrolysis of triacetin in the presence of a chloride source also generated compound **2**. One of the advantages of utilizing a short-chain CP diester **2** as a model is that it maximizes the samples' volatility while minimizing its thermal degradation,[10] thus enhancing mass spectral signals of the characteristic peaks especially under hard ionization conditions such as EI. When compound **2** was analyzed under EI conditions, it indeed generated an intense ion at m/z 135.6 consistent with the molecular mass of the corresponding diacylglyceride ion. As expected, the isotope labeling studies of 1,2-bis(acetoyl)-3-chloropropane (**2**) (see Table 3) indicated the incorporation of three carbon atoms from glycerol and four carbon atoms from acetic anhydride, and the ion at m/z 135.6 incorporated only two carbon atoms from acetic anhydride and three carbon atoms from glycerol, confirming their respective structures. Furthermore, the major ion in question can also be generated from a commercially available 4-chloromethyl-2,2-dimethyl-1,3-dioxolane (**3**) under EI conditions by the loss of a methyl radical as shown in Figure 2. Therefore, MS^2 analysis of daughter ions of m/z 135.6 generated from **2** and **3** should provide adequate information regarding their similarity. However, due to the stability of the ion at m/z 135 only a very weak daughter ion at m/z 75 was generated from both precursors, nevertheless confirming their similarity. According to Eberlin and Cooks[19] the formation of m/z 75 is characteristic of a dioxolane moiety. The identical tandem mass spectra obtained from ions at m/z 135.6 originating from compounds **2** and **3** establish that the two fragment ions have identical structures. Moreover, the structure of m/z 135.6 originating from inherently cyclic 4-chloromethyl-2,2-dimethyl-

dx.doi.org/10.1021/jf401118n | J. Agric. Food Chem. 2013, 61, 5946−5952

1,3-dioxolane by a loss of methyl radical should retain the original cyclic nature of the parent ion, thus confirming the structure of m/z 135.6 originating from 1,2-bis(acetoyl)-3-chloropropane (**2**) as a cyclic moiety. Furthermore, as shown in Table 2, the compound 1,2-bis(acetoyl)-3-chloropropane (**2** in Figure 2) also generated the ion at m/z 135 under ESI and APCI conditions. Interestingly, when 3-MCPD diesters containing longer chain fatty acids such as 1-palmitoyl-2-stearoyl-3-chloropropane or 1,2-dipalmitoyl-3-chloropropane were studied using EI conditions, masses consistent with McLafferty rearrangement products (structure **4**) were observed at m/z 419 and 390, respectively (details will be published elsewhere). Furthermore, these monoacetyl derivatives of CP diesters (**4** in Figure 2) can also undergo displacement rearrangement under EI conditions, generating the identical m/z 135.6 ions as depicted in Figure 2. Ion series corresponding to the structure **4** have already been observed in the EI spectra of many saturated TAGs.[20] Such ions could hold a great potential as a convenient screening tool under EI conditions for the presence of CP esters by monitoring a single mass at m/z 135.

Implications of $[M - RCO_2]^+$ Ions in Lipid Chemistry. Because $[M - RCO_2]^+$ ions can be formed as unprotonated cationic species under various ionization conditions in both acylglycerols and CP esters, they may be considered as universal indicators or diagnostic ions for the presence of CP-diesters during GC-MS or LC-MS analysis. AA type CP diesters, for example, should generate one acyloxonium ion (A^+) and AB type CP diesters should generate two acyloxonium ions (A^+ and B^+), with the most intense ion being generated by the loss of either sn-1 or sn-3 fatty acid.[21] In fact, Zelinková et al.[13] have already used such ions to identify 3-MCPD diesters under EI conditions. CP monoesters, on the other hand, generate only low-intensity cyclic acyloxonium ions and cannot be used reliably as diagnostic ions compared with their diester counterparts. Furthermore, cyclic acyloxonium ions have been proposed by various groups[12] to form in heated oils as reactive intermediates leading to the formation of 3-MCPD diesters in the presence of a chloride source. Spectroscopic evidence for the thermal formation of cyclic acyloxonium ions under acid catalysis in heated tripalmitin has been provided by Rahn and Yaylayan[22] using ATR FTIR analysis. The appearance of a temperature-dependent new band at 1650 cm^{-1} in the heated samples suggested the formation of cyclic acyloxonium ions consistent with the absorption band of cyclic dioxoles reported in the literature.[23] As expected, the band centered at 1650 cm^{-1} shifted to 1610 cm^{-1} when isotopically labeled (1,1,1-$^{13}C_3$) tripalmitin was used instead, confirming the involvement of the carboxylic acid carbon atoms in the evolution of the band at 1650 cm^{-1}. A similar band was observed by Nichols and Holmes[24] from a neat triacylglycerol sample pyrolyzed at 600 °C, allowing them to conclude that this compound contained a cyclic structure. Such a similarity between thermally generated structures and those formed under EI conditions is not surprising because there is ample evidence in the literature supporting such correspondence.[24−28] Consequently, thermally generated counterparts of structure **4** or even salt-stabilized counterparts of m/z 135.6 may serve as convenient chemical markers for 3-MCPD diesters in refined edible oils.

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Funding

We acknowledge funding for this research from the Natural Sciences and Engineering Research Council of Canada (NSERC).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Kalo, P. J.; Ollilainen, V.; Rocha, J. M.; Malcata, F. X. Identification of molecular species of simple lipids by normal phase liquid chromatography-positive electrospray tandem mass spectrometry, and application of developed methods in comprehensive analysis of low erucic acid rapeseed oil lipids. *Int. J. Mass Spectrom.* **2006**, *254*, 106−121.

(2) McAnoy, A. M.; Wu, C. C.; Murphy, R. C. Direct qualitative analysis of triacylglycerols by electronspray mass spectrometry using linear ion trap. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 1498−1509.

(3) Mottram, H. R.; Woodbury, S. E.; Evershed, R. P. Identification of triacylglycerol positional isomers present in vegetable oils by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1240−1252.

(4) Cheng, C.; Gross, M. L.; Pittenauer, E. Complete structural elucidation of triacylglycerols by tandem sector mass spectrometry. *Anal. Chem.* **1998**, *70*, 4417−4426.

(5) Kim, Y. H.; So, K.-Y.; Limb, J.-K.; Jhon, G.-J.; Han, S.-Y. Identification of triacylglycerols containing two short-chain fatty acids at sn-2 and sn-3 positions from bovine udder by fast atom bombardment tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 2230−2237.

(6) Marzilli, L. A.; Fay, L. B.; Dionisi, F.; Vouros, P. Structural characterization of triacylglycerols using electrospray ionization-MS^n ion-trap MS. *J. Am. Oil Chem. Soc.* **2003**, *80*, 195−202.

(7) Laakso, P. Analysis of triacylglycerols − approaching the molecular composition of natural mixtures. *Food Rev. Int.* **1996**, *12*, 199−250.

(8) Mu, H.; Sillen, H.; Hıy, C.-E. Identification of diacylglycerols and triacylglycerols in a structured lipid sample by atmospheric pressure chemical ionization liquid chromatography/mass spectrometry. *J. Am. Oil Chem. Soc.* **2000**, *77*, 1049−1060.

(9) Smith, M. B.; March, J. Chapter 10: Aliphatic substitution: nucleophilic and organometallic. In *March's Advanced Organic Chemistry: Reactions, Mechanisms, And Structure, 6*; Smith, M. B., March, J., Eds.; Wiley: Hoboken, NJ, 2007; p 501.

(10) Duffin, K. L.; Henion, J. D.; Shieh, J. J. Electrospray and tandem mass spectrometric characterization of acylglycerol mixtures that are dissolved in nonpolar solvents. *Anal. Chem.* **1991**, *63*, 1781−1788.

(11) Velíšek, J.; Davídek, J.; Hajšlová, J.; Kubelka, V.; Janíček, G.; Mánková, B. Chlorohydrins in protein hydrolysates. *Z. Lebensm. Unters. Forsch.* **1978**, *167*, 241−244.

(12) Destaillats, F.; Craft, B. D.; Sandoz, L.; Nagy, K. Formation mechanisms of monochloropropanediol (MCPD) fatty acid diesters in refined palm (*Elaeis guineensis*) oil and related fractions. *Food Addit. Contam.* **2012**, *29*, 29−37.

(13) Zelinková, Z.; Doležal, M.; Velíšek, J. Occurrence of 3-chloropropane-1,2-diol fatty acid esters in infant and baby foods. *Eur. Food Res. Technol.* **2009**, *228*, 571−578.

(14) Murphy, R. C.; Fiedler, J.; Hevko, J. Analysis of nonvolatile lipids by mass spectrometry. *Chem. Rev.* **2001**, *101*, 479−526.

(15) Schiller, J.; Süβ, R.; Arnhold, J.; Fuchs, B.; Leβig, J.; Müller, M.; Petković, M.; Spalteholz, H.; Zschörnig, O.; Arnold, K. Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass

spectrometry in lipid and phospholipid research. *Prog. Lipid Res.* **2004**, *43*, 449−488.
(16) Herrera, L. C.; Potvin, M. A.; Melanson, J. E. Quantitative analysis of positional isomers of triacylglycerols via electrospray ionization tandem mass spectrometry of sodiated adducts. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2745−2752.
(17) Emerson, B.; Gidden, J.; Lay, J. O.; Durham, B. Laser desorption/ionization time-of-flight mass spectrometry of triacylglycerols and other components in fingermark samples. *J. Forensic Sci.* **2011**, *56*, 381−389.
(18) Gerbig, S.; Takáts, Z. Analysis of triglycerides in food items by desorption electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2186−2192.
(19) Eberlin, M. N.; Cooks, R. G. Gas-phase oxirane addition to acylium ions on reaction with 1,3-dioxolanes elucidated by tandem and triple stage mass spectrometric experiments. *Org. Mass Spectrom.* **1993**, *28*, 679−687.
(20) Lauer, W. M.; Aasen, A. J.; Graff, G.; Holman, R. T. Mass spectrometry of triglycerides: I. Structural effects. *Lipids* **1970**, *5*, 861−868.
(21) Mottram, H. R.; Evershed, R. P. Structure analysis of triacylglycerol positional isomers using atmospheric pressure chemical ionization mass spectrometry. *Tetrahedron Lett.* **1996**, *37*, 8593−8596.
(22) Rahn, A. K. K.; Yaylayan, V. A. Monitoring cyclic acyloxonium ion formation in palmitin systems using infrared spectroscopy and isotope labelling technique. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 330−334.
(23) Alonso, M. E.; Jano, P. The syntheses of ethoxycarbonyl-1,3-dioxoles and oxazoles from the copper catalyzed thermolysis of ethyl diazopyruvate in the presence of ketones, aldehydes and nitriles. *J. Heterocycl. Chem.* **1980**, *17*, 721−725.
(24) Nichols, P. C.; Holman, R. T. Pyrolysis of saturated triglycerides. *Lipids* **1972**, *7*, 773−779.
(25) Moldoveanu, S. C. Pyrolysis process compared to ion fragmentation in mass spectrometry. In *Analytical Pyrolysis of Natural Organic Polymers*; Moldoveanu, S. C., Ed.; Elsevier Science: Amsterdam, The Netherlands, 1998; Vol. 20, pp 55−66.
(26) Holmes, J. L.; Aubry, C.; Mayer, P. M. Tools for identifying the structure of gas-phase ions: thermochemistry; its role in assigning ion structures. In *Assigning Structures to Ions in Mass Spectrometry*; Holmes, J. L., Aubry, C., Mayer, P. M., Eds.; Taylor and Francis Group: Boca Raton, FL, 2007; pp 15−36.
(27) Murphy, R. C. Triacylglycerols, diacylglycerols, and monoacylglycerols. In *Handbook of Lipid Research 7: Mass Spectrometry of Lipids*; Murphy, R. C., Ed.; Plenum Press: New York, 1993; pp 189−210.
(28) Butovich, I. A.; Uchiyama, E.; McCulley, J. P. Lipids of human meibum: mass-spectrometric analysis and structural elucidation. *J. Lipid Res.* **2007**, *48*, 2220−2235.