$O^{-\cdot}$ and OH^- Chemical Ionization of Some Fatty Acid Methyl Esters and Triacylglycerols

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$O^{-\cdot}$ and OH^- react with fatty acid methyl esters (FAMES) under chemical ionization conditions both as Brønsted bases to form $[M - H]^-$ and as nucleophiles to form the carboxylate ion $RCOO^-$. $O^{-\cdot}$ shows a much greater tendency to react as a nucleophile than does OH^-. The $[M - H]^-$ ions fragment by elimination of CH_3OH, with unsaturation in certain positions in the fatty acid hydrocarbon chain promoting this elimination for unknown reasons. The reaction of $O^{-\cdot}$ and OH^- with triacylglycerols leads to $[M - H]^-$, characteristic of the molecular mass, and to carboxylate ions characteristic of the fatty acid(s) present in the lipid. The presence of the three ester functions in the lipids greatly enhances the formation of carboxylate ions compared to the FAMES. *(J Am Soc Mass Spectrom 1994, 5, 553–557)*

Although the initial studies in chemical ionization (CI) were concerned exclusively with positive ion CI [1, 2], the interest and activity in negative ion CI have grown significantly over the past 15 years [3–5]. In negative ion CI, Brønsted bases, B^-, play a role analogous to the role played by Brønsted acids in positive ion CI. Frequently, the dominant reaction of B^- is proton abstraction to form $[M - H]^-$, thus providing information as to molecular mass but little structural information. The oxide radical anion, $O^{-\cdot}$, is unusual among negative ion CI reagent ions in that not only is it a strong Brønsted base, but also it is a radical. As a result, it reacts with many organic substrates, not only by proton abstraction, but also by H atom abstraction, by $H_2^{+\cdot}$ abstraction, and by H atom and alkyl radical displacement [6–20]. This variety of pathways creates the possibility that $O^{-\cdot}$ CI can provide more structural information than other negative ion reagents, and this has been demonstrated in a number of studies [8–13].

In a continuation of our studies [12, 13, 18, 20] of $O^{-\cdot}$ CI of organic molecules, we report here the $O^{-\cdot}$ CI mass spectra of a selection of fatty acid methyl esters (FAMES) and triacylglycerols. Because of the importance of fatty acids and their derivatives, such as lipids, in biological systems [21], there have been many studies of the electron ionization (EI) and positive ion CI mass spectra of these classes of compounds [22–27], although suprisingly few studies in the negative ion field. In a limited study, Bambagiotti et al. [28, 29] have reported the OH^- CI mass spectra of a few FAMES and observed abundant $[M - H]^-$ ions, characteristic of molecular mass, as well as carboxylate ions arising by nominal methyl radical elimination from the molecular anion. In a very brief article [30], it has been noted that the OH^- CI mass spectrum of mixed fatty acid esters of chloropropandiol shows low-intensity $[M - H]^-$ ions characteristic of the molecular masses, as well as intense carboxylate ions identifying the carboxylic acids present in the mixed esters; this result suggests that OH^- or $O^{-\cdot}$ CI of triacylglycerols might be a useful analytical approach. In the present study we have also recorded the OH^- CI mass spectra of the FAMES and triacylglycerols for comparative purposes.

Experimental

Mass spectra were obtained using a VG 70-250S EB double focusing mass spectrometer (VG Analytical Ltd., Manchester, England) which was linked to a VG 11-250J data system. The source temperature was 250 °C, the ion chamber pressure gauge reading was 7.5×10^{-5} torr (which corresponded to 0.1–0.3 torr pressure in the ion source), the ionizing electron energy was 100 eV, and the ion accelerating voltage was 6 kV. The FAMES were introduced into the ion source by way of an HP5890A gas chromatograph (Hewlett-Packard, Avondale, PA), operating with a 30-m DB5 capillary column. The initial temperature was 190 °C with a ramp of 20 °/min to a final temperature of 270 °C. Approximately 200 ng of sample was injected onto the column. The triacylglycerols were introduced into the source by a direct exposure probe, except for triacetin and tributyrin which were introduced using a direct insertion probe. The CI mass spectra of the FAMES also were obtained using a VG Analytical ZAB-2FQ (BEqQ) mass spectrometer [31] operating in the double focusing mode with introduction of the ester either by a heated inlet system or by a direct insertion probe.

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The operating conditions were the same as for the 70-250S instrument.

The OH^- reagent ions were prepared by electron bombardment of a 10% N_2O/90% CH_4 mixture, while the $O^{-\cdot}$ reagent ions were prepared by electron bombardment of a 10% N_2O/90% N_2 mixture.

High-purity (99% by gas chromatography and thin-layer chromatography) fatty acid methyl esters and triacylglycerols were purchased from Sigma Chemical Co. (St. Louis, MO) and were used as received.

Results and Discussion

Fatty Acid Methyl Esters

Tables 1 and 2 present the $O^{-\cdot}$ and OH^- CI mass spectra of the C6–C20 FAMES, including a variety of C18 and C20 unsaturated esters. With both reagents three ion species are observed, $[M - H]^-$, $RCOO^-$, and $[M - H - CH_3OH]^-$. The latter ion arises by elimination of CH_3OH from the $[M - H]^-$ ion as shown by collision-induced dissociation studies [29, 32, 33]. Labeling studies [29] with methyl hexanoate have shown that a hydrogen alpha to the carbonyl function is abstracted to form $[M - H]^-$, and that the second α-hydrogen is abstracted in the collision-induced methanol-loss reaction. In their initial report of the OH^- CI mass spectra of methyl esters of C14–C18 unsaturated fatty acids, Bambagiotti et al. [28] listed $[M - H - CH_3OH]^-$ ions only for the polyunsaturated compounds; however, in their more recent article [29] on the OH^- CI mass spectra of the methyl esters of C18 saturated and mono-unsaturated fatty acids they report low-abundance $[M - H - CH_3OH]^-$ ion signals for all esters. We have observed that the $[M - H - CH_3OH]^-$ ion abundances were greater when the mass spectra were determined using the 70-250S mass spectrometer than when determined using the ZAB-2F mass spectrometer; the reasons for this difference are not understood, but it appears that the abundance of this fragment ion depends on undefined experimental conditions. A number of the unsaturated FAMES show a particularly pronounced abundance of the $[M - H - CH_3OH]^-$ fragment ion in both the $O^{-\cdot}$ and OH^- CI mass spectra. These include C18:1(*cis*-6), C18:3(*cis*-6,9,12), C20:2(*cis*-11,14), and C20:3(*cis*-5,8,11). No clear pattern emerges from these results. Thus, although the C18:1(*cis*-6) ester shows an enhanced $[M - H - CH_3OH]^-$ ion signal, the C20:1(*cis*-5) ester does not. It appears that there are subtle conformational effects which influence the extent of CH_3OH elimination from $[M - H]^-$. Clearly, it would be of interest to study a complete series of mono-unsaturated and di-unsaturated esters; however, these are not available. In addition, deuterium labeling to confirm that CH_3OH elimination exclusively involves the α-hydrogen in these cases would be of interest, but is difficult experimentally.

Table 1. $O^{-\cdot}$ CI Mass Spectra of $RCOOCH_3$

RCO	*m/z* (Relative Abundance) $[M - H]^-$	$RCOO^-$	$[M - H - MeOH]^-$
C6:0	129 (54)	115 (100)	95 (5)
C8:0	157 (61)	143 (100)	125 (7)
C10:0	185 (63)	171 (100)	153 (8)
C12:0	213 (68)	199 (100)	181 (11)
C14:0	241 (61)	227 (100)	209 (12)
C16:0	269 (51)	255 (100)	237 (14)
C18:0	297 (54)	283 (100)	265 (15)
C18:1(*cis*-6)	295 (86)	281 (100)	263 (89)
C18:1(*cis*-9)	295 (100)	281 (76)	263 (8)
C18:1(*cis*-11)	295 (100)	281 (66)	263 (8)
C18:2(*cis*-9,12)	293 (100)	279 (80)	261 (50)
C18:3(*cis*-6,9,12)	291 (8)	277 (56)	259 (100)
C18:3(*cis*-9,12,15)	291 (100)	277 (43)	259 (15)
C20:1(*cis*-5)	323 (100)	309 (42)	291 (18)
C20:1(*cis*-8)	323 (100)	309 (33)	291 (13)
C20:1(*cis*-11)	323 (100)	309 (28)	291 (12)
C20:1(*cis*-13)	323 (100)	309 (29)	291 (14)
C20:2(*cis*-11,14)	321 (58)	307 (31)	289 (100)
C20:3(*cis*-5,8,11)	319 (3)	305 (23)	287 (100)
C20:3(*cis*-8,11,14)	319 (100)	305 (23)	287 (19)
C20:3(*cis*-11,14,17)	319 (100)	305 (32)	287 (36)

Table 2. OH^- CI Mass Spectra of $RCOOCH_3$

RCO	*m/z* (Relative Abundance) $[M - H]^-$	$RCOO^-$	$[M - H - MeOH]^-$
C6:0	129 (100)	115 (13)	97 (6)
C8:0	157 (100)	143 (13)	125 (10)
C10:0	185 (100)	171 (13)	153 (12)
C12:0	213 (100)	199 (11)	181 (14)
C14:0	241 (100)	227 (12)	209 (20)
C16:0	269 (100)	255 (10)	237 (20)
C18:0	297 (100)	283 (9)	265 (22)
C18:1(*cis*-6)	295 (100)	281 (14)	263 (82)
C18:1(*cis*-9)	295 (100)	281 (9)	263 (9)
C18:1(*cis*-11)	295 (100)	281 (8)	263 (9)
C18:2(*cis*-9,12)	293 (100)	279 (8)	261 (45)
C18:3(*cis*-6,9,12)	291 (10)	277 (7)	259 (100)
C18:3(*cis*-9,12,15)	291 (100)	277 (7)	259 (15)
C20:1(*cis*-5)	323 (100)	309 (10)	291 (20)
C20:1(*cis*-8)	323 (100)	309 (7)	291 (14)
C20:1(*cis*-11)	323 (100)	309 (7)	291 (13)
C20:1(*cis*-13)	323 (100)	309 (7)	291 (14)
C20:2(*cis*-11,14)	321 (54)	307 (8)	289 (100)
C20:3(*cis*-5,8,11)	319 (4)	305 (6)	287 (100)
C20:3(*cis*-8,11,14)	319 (100)	305 (5)	287 (20)
C20:3(*cis*-11,14,17)	319 (100)	305 (6)	287 (34)

It is well established [34–39], from the reaction of $^{18}O^{-\cdot}$ and $^{18}OH^-$ with formate, trifluoroacetate, and benzoate esters, that the carboxylate ions arise by both an S_N2 and a $B_{AC}2$ reaction, the latter involving nucleophilic attack at the carbonyl carbon. Using the ratio $([M - H]^- + [M - H - CH_3OH]^-)/[RCOO]^-$ as a measure of proton abstraction versus nucleophilic attack (both S_N2 and $B_{AC}2$), we note for $O^{-\cdot}$ reacting with saturated FAMES a ratio of $\sim 0.7 \pm 0.1$ compared to a ratio of 10 ± 2 for the reaction of OH^-. It is clear that $O^{-\cdot}$ has a much greater tendency to react as

a nucleophile than OH^-. One also should note that in the $O^{-\cdot}$ CI mass spectra the ratio increases substantially for unsaturated FAMES (to a value in the range 1.4–5.1). This raises the possibility that $O^{-\cdot}$ may be abstracting a proton from positions allylic to the double bond(s). Gas phase acidity data for olefins are scarce; however, we note that $\Delta H°_{acid}$(cyclohexene) = 1617 kJ mol^{-1} [40] compared to $\Delta H°_{acid}$ (OH) = 1599 kJ mol^{-1} [40]; since our reactant ions are not thermally equilibrated but probably have excess kinetic energy [19, 20], proton abstraction from allylic positions in the unsaturated FAMES is distinctly possible. Since $\Delta H°_{acid}(H_2O)$ = 1635 kJ mol^{-1} [40], proton abstraction from allylic positions by OH^- probably is occurring. However, since H^+-abstraction is the dominant reaction under OH^- CI conditions, the effect of this reaction channel is not clearly reflected in the relative ion abundances. Again, deuterium labeling would be useful, but is difficult. It is interesting to note that no $[M - 2H]^{-\cdot}$ ions are observed in the reaction of $O^{-\cdot}$ with the esters, although this is a significant reaction channel with many ketones [13].

For the same sample size introduced, the total sample ion signals were four to ten times greater in the OH^- CI mass spectra than in the $O^{-\cdot}$ CI mass spectra. We have observed that, in the absence of sample, the signal for $O^{-\cdot}$ in the N_2/N_2O mixture is less than that of OH^- in the CH_4/N_2O mixture. This probably reflects the lower efficiency of N_2 relative to CH_4 in thermalizing electrons [41, 42]. In addition, $O^{-\cdot}$ reacts with the esters partly by H-atom abstraction to give OH^-, which also will lower the sensitivity for detection of the ester under $O^{-\cdot}$ CI conditions. In line with this observation we noted that when the FAME was introduced using a direct inlet probe (which resulted in a higher partial pressure of the ester in the source), the $[M - H]^-$ ion signal was enhanced in the $O^{-\cdot}$ CI mass spectra due to the occurrence of the reaction sequence

$$O^{-\cdot} + M \rightarrow OH^- + [M - H] \quad (1)$$

$$OH^- + M \rightarrow [M - H]^- + H_2O \quad (2)$$

As expected, the OH^- CI mass spectra were independent of sample size over the limited range investigated.

Triacylglycerols

Table 3 records the $O^{-\cdot}$ and OH^- CI mass spectra of a series of triacylglycerols in which the three acyl groups are identical. Although triacetin and tribuytrin do not qualify as triacylglycerols of fatty acids, they have been included for completeness. Only two ionic products are observed in the spectra, the $[M - H]^-$ ion and the carboxylate ion $RCOO^-$. Specifically, no fragment ion analogous to the $[M - H - CH_3OH]^-$ ion observed for the FAMES is observed for the triacylglycerols. The carboxylate ion $RCOO^-$ is much more abundant for the triacylglycerols than it is for the corresponding FAME. Thus, for the OH^- CI mass spectrum of tripalmitin, $(CH_2(OCOC_{15}H_{31})$-$CH(OCOC_{15}H_{31})$-$CH_2(OCOC_{15}H_{31}))$, $[M - H]^-/[RCOO]^- = 0.25$, while for methyl palmitate, (C16:0) $([M - H]^- + [M - H - CH_3OH]^-)/[RCOO]^- = 12$. Clearly, the triester functionality has greatly increased the extent of nucleophilic attack relative to proton abstraction in reaction of both $O^{-\cdot}$ and OH^-. In addition, as observed in the reaction of FAMES, $O^{-\cdot}$ has a greater tendency to react as a nucleophile than does OH^-. It would be of interest to explore the extent of carboxylate ion formation by S_N2 and $B_{AC}2$ reactions in these cases; since this would involve the use of $^{18}O^{-\cdot}$ and $^{18}OH^-$ reagent ions, the experiments are best carried out using Fourier transform mass spectrometry.

Table 3. $O^{-\cdot}$ and OH^- CI Mass Spectra of Triacylglycerols $CH_2(OCOR)$-$CH(OCOR)$-$CH_2(OCOR)$

	m/z (Relative Abundance)			
Triacylglycerol	$O^{-\cdot}$ CI $[M - H]^-$	$RCOO^-$	OH^- CI $[M - H]^-$	$RCOO^-$
Triacetin	217 (90)	59 (100)	217 (100)	59 (74)
Tributyrin	301 (31)	87 (100)	301 (48)	87 (100)
Tricaproin	385 (8)	115 (100)	385 (46)	115 (100)
Trilaurin	637 (7)	199 (100)	637 (23)	199 (100)
Trimyristin	721 (7)	227 (100)	721 (26)	227 (100)
Tripalmitin	805 (12)	255 (100)	805 (25)	255 (100)
Tristearin	889 (12)	283 (100)	889 (11)	283 (100)
Triolein	883 (7)	281 (100)	883 (15)	281 (100)

Tables 4 and 5 present, respectively, the $O^{-\cdot}$ and OH^- CI mass spectra of triacylglycerols containing two different fatty acids. (The designation C18:1 corresponds to oleic acid which has a *cis*-9 double bond.) Three ion signals are observed, $[M - H]^-$ and the carboxylate ions derived from the two different fatty acids. In all cases the base peak corresponds to the carboxylate ion derived from the fatty acid which has a double presence in the lipid. The second carboxylate ion has an abundance which is 40–80% of the base peak. The first six entries in the tables are triacylglycerols which can be represented as A/A/B. The last eight entries represent isomeric pairs A/A/B and A/B/A where the second fatty acid (B) is in the 3 position (A/A/B) or in the 2 position (A/B/A) of the glycerol. There are not sufficient differences in the spectra of the isomeric pairs to distinguish the site of attachment of the fatty acids to the glycerol. The results do serve to identify the molecular mass of the lipid through the $[M - H]^-$ ion and to identify the

Table 4. $O^{-\cdot}$ CI Mass Spectra of Triacylglycerols $CH_2(OCOR_1)$-$CH(OCOR_2)$-$CH_2(OCOR_3)$

	m/z (Relative Abundance)			
R_1CO /R_2CO /R_3CO	$[M-H]^-$	R_1COO^-	R_2COO^-	R_3COO^-
C12:0 /C12:0 /C14:0	665 (8)	199 (100)	R_1COO^-	227 (60)
C14:0 /C14:0 /C12:0	693 (9)	227 (100)	R_1COO^-	199 (58)
C14:0 /C14:0 /C16:0	749 (8)	227 (100)	R_1COO^-	255 (60)
C16:0 /C16:0 /C14:0	777 (10)	255 (100)	R_1COO^-	227 (63)
C18:0 /C18:0 /C14:0	833 (7)	283 (100)	R_1COO^-	227 (65)
C18:0 /C18:0 /C16:0	861 (13)	283 (100)	R_1COO^-	255 (57)
C16:0 /C16:0 /C18:1	831 (7)	255 (100)	R_1COO^-	281 (46)
C16:0 /C18:1 /C16:0	831 (5)	255 (100)	281 (45)	R_1COO^-
C18:1 /C18:1 /C16:0	857 (11)	281 (100)	R_1COO^-	255 (64)
C18:1 /C16:0 /C18:1	857 (13)	281 (100)	255 (47)	R_1COO^-
C18:1 /C18:1 /C18:0	885 (7)	281 (100)	R_1COO^-	283 (73)
C18:1 /C18:0 /C18:1	885 (15)	281 (100)	283 (73)	R_1COO^-
C18:0 /C18:0 /C18:1	887 (12)	283 (100)	R_1COO^-	281 (57)
C18:0 /C18:1 /C18:0	887 (12)	283 (100)	281 (60)	R_1COO^-

Table 5. OH^- CI Mass Spectra of Triacylglycerols $CH_2(OCOR_1)$-$CH(OCOR_2)$-$CH_2(OCOR_3)$

	m/z (Relative Abundance)			
R_1CO /R_2CO /R_3CO	$[M-H]^-$	R_1COO^-	R_2COO^-	R_3COO^-
C12:0 /C12:0 /C14:0	665 (32)	199 (100)	R_1COO^-	227 (68)
C14:0 /C14:0 /C12:0	693 (30)	227 (100)	R_1COO^-	199 (56)
C14:0 /C14:0 /C16:0	749 (28)	227 (100)	R_1COO^-	255 (68)
C16:0 /C16:0 /C14:0	777 (32)	255 (100)	R_1COO^-	227 (54)
C18:0 /C18:0 /C14:0	833 (27)	283 (100)	R_1COO^-	227 (54)
C18:0 /C18:0 /C16:0	861 (17)	283 (100)	R_1COO^-	255 (64)
C16:0 /C16:0 /C18:1	831 (34)	255 (100)	R_1COO^-	281 (52)
C16:0 /C18:1 /C16:0	831 (17)	255 (100)	281 (44)	R_1COO^-
C18:1 /C18:1 /C16:0	857 (24)	281 (100)	R_1COO^-	255 (66)
C18:1 /C16:0 /C18:1	857 (30)	281 (100)	255 (43)	R_1COO^-
C18:1 /C18:1 /C18:0	885 (26)	281 (100)	R_1COO^-	283 (80)
C18:1 /C18:0 /C18:1	885 (41)	281 (100)	283 (72)	R_1COO^-
C18:0 /C18:0 /C18:1	887 (30)	283 (100)	R_1COO^-	281 (64)
C18:0 /C18:1 /C18:0	887 (27)	283 (100)	281 (48)	R_1COO^-

fatty acids present through the carboxylate ions formed. Again, $O^{-\cdot}$ reacts more as a nucleophile than as a Brønsted base, with the result that the $[M-H]^-$ ions, characteristic of molecular mass, are of relatively low intensity in the $O^{-\cdot}$ CI mass spectra.

Conclusions

The negative ion reagents $O^{-\cdot}$ and OH^- react with FAMES both as a Brønsted base to form $[M-H]^-$ and as a nucleophile to form $RCOO^-$ ($[M-CH_3]^-$). $O^{-\cdot}$ shows a greater tendency to react as a nucleophile than does OH^-. The $[M-H]^-$ ions, characteristic of molecular mass, fragment by loss of CH_3OH, and in some cases this is a major reaction channel, although the factors favoring this reaction are not clear. The reaction of $O^{-\cdot}$ and OH^- with triacylglycerols leads to $[M-H]^-$, indicative of the molecular mass, and to carboxylate ions characterizing the fatty acids present in the lipid. This work thus confirms the earlier brief article [30] suggesting that negative ion CI of lipids would be a useful analytical approach. The spectra obtained do not provide any indication of the position of unsaturation in the fatty acid component. However, it has been shown that high-energy collision-induced dissociation of the $[M-H]^-$ ions of FAMES [28, 29] or the carboxylate ions derived from fatty acids [43–45] does provide information on the position(s) of unsaturation.

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