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Journal of Chromatography A, 1061 (2004) 115–121
JOURNAL OF CHROMATOGRAPHY A
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Short communication

Dicarboxylic degradation products of nonylphenol polyethoxylates: synthesis and identification by gas chromatography–mass spectrometry using electron and chemical ionization modes

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Received 5 August 2004; received in revised form 23 September 2004; accepted 11 October 2004

Abstract

The synthesis, mass spectra and detectability of four selected dicarboxylic degradation products (CAPECs) of nonylphenol polyethoxylates (NPEOs) are reported. The selected isomers have an α,α-dimethyl configuration (expressed as "dm" in their abbreviation), five to eight C atoms and a carboxyl group in the alkyl chain, and a carboxymethoxy acid group (dm-$CA_{5–8}$P1ECs). The synthesis was successfully accomplished via a reaction sequence that started from anisole. After trimethylsilylation with *N*,*O*-bis(trimethylsilyl)acetamide or methylation with (trimethylsilyl)diazomethane, the derivatives of the dm-$CA_{5–8}$P1ECs were subjected to a GC–electron ionization (EI)–MS and GC–isobutane chemical ionization (CI)–MS. In EI–MS, ion peaks at $m/z = 265$ and 207, corresponding to the α,α-dimethyl structures via the benzyl cleavage of carboxyalkyl chain, were the most significant ions of the trimethylsilyl and methyl derivatives, respectively. In CI–MS, the main ion peaks of dm-CA_5-, dm-CA_6-, dm-CA_7-, and dm-CA_8P1EC after methylation were at $m/z = 129$, 143, 157, and 171, respectively, corresponding to the loss of methyl phenoxyacetate from $[M + H]^+$; meanwhile significant peaks were detected at 321, 335, 349, and 363, corresponding to the loss of the trimethylsilanol after trimethylsilylation. The potential for the identification and quantification of individual branched carboxyalkyl isomeric mixtures of CA_5-, CA_6-, CA_7-, and CA_8P1EC metabolites based on corresponding dm-$CA_{5–8}$P1ECs revealed the advantage of the GC–CI–MS although the detection limits in CI were clearly higher than those in EI.

Keywords: Dicarboxylic degradation products; Derivatization; Environmental analysis; Nonylphenol polyethoxylates; GC

1. Introduction

The dicarboxylic degradation products (CAPECs) of nonylphenol polyethoxylates (NPEOs) have been recently recognized to occur in different environmental compartments [1–10] at relatively high, microgram per liter, levels [1–6,9]. Particularly, those CAPECs that contain five to eight C atoms and a carboxyl group in the alkyl chain are believed to be the most commonly occurring metabolites in an aqueous environment as they appeared to be extremely persistent species (remain even after 5 months following their generation on laboratory scale wastewater treatment unit [3]), and accounted for between 60% and 63% of the total pool of degradation metabolites in river water and treatment plant effluents, respectively [4,6]. Of the CAPECs class, the carboxyalkylphenol monoethoxycarboxylates (CAP1ECs) [2,3] and carboxyalkylphenol diethoxycarboxylates (CAP2ECs) [5–7] are the most common.

The relatively high concentration and possible persistence of the CAPECs in the environment may be reasons for concern; however, the identification and quantification of these

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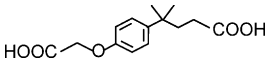
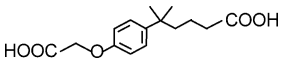
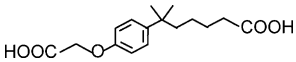
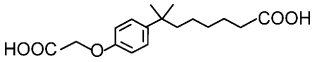
doi:10.1016/j.chroma.2004.10.041

Table 1
Structures, abbreviations, and ^{1}H and ^{13}C NMR spectra of $CA_{5–8}P1ECs$ synthesized in this study

No.	cChemical name	Abbreviation	Structure	^{1}H NMR (CD_3OD) δ	^{13}C NMR (CD_3OD) δ
1	4-(4′-Carboxy-2′-methyl-2′-butyl)phenoxyacetic acid	dm-CA_5P1EC		1.29 (s, 6H), 1.89–1.95 (m, 2H), 1.97–2.03 (m, 2H), 2.19 (t, J = 7.43 Hz, 2H), 4.63 (s, 2H), 6.88 (d, J = 9.06 Hz, 2H), 7.27 (d, J = 9.06 Hz, 2H)	29.35, 31.03, 37.76, 40.34, 65.99, 115.39, 128.00, 142.45, 157.49, 172.92, 177.93
2	4-(5′-Carboxy-2′-methyl-2′-pentyl)phenoxyacetic acid	dm-CA_6P1EC		1.28 (s, 6H), 1.29–1.39 (m, 2H), 1.59–1.65 (m, 2H), 2.17 (t, J = 7.43 Hz, 2H), 4.62 (s, 2H), 6.86 (d, J = 9.06 Hz, 2H), 7.26 (d, J = 9.06 Hz, 2H)	21.64, 29.63, 35.47, 38.02, 45.05, 66.03, 115.26, 127.97, 143.34, 157.32, 172.99, 177.57
3	4-(6′-Carboxy-2′-methyl-2′-hexyl)phenoxyacetic acid	dm-CA_7P1EC		1.03–1.12 (m, 2H), 1.26 (s, 6H), 1.49 (quint, J = 7.61 Hz, 2H), 1.57–1.63 (m, 2H), 2.19 (t, J = 7.61 Hz, 2H), 4.62 (s, 2H), 6.85 (d, J = 9.06 Hz, 2H), 7.25 (d, J = 9.06 Hz, 2H)	25.53, 26.77, 29.66, 34.92, 38.03, 45.40, 66.01, 115.21, 127.94, 143.58, 157.25, 172.97, 177.65
4	4-(7′-Carboxy-2′-methyl-2′-heptyl)phenoxyacetic acid	dm-CA_8P1EC		1.00–1.11 (m, 2H), 1.19–1.29 (m, 8H including s (1.26 ppm, 6H)), 1.50 (quint, J = 7.61 Hz, 2H), 1.56–1.62 (m, 2H), 2.19 (t, J = 7.43 Hz, 2H), 4.62 (s, 2H), 6.85 (d, J = 9.06 Hz, 2H), 7.25 (d, J = 9.06 Hz, 2H)	25.60, 26.00, 29.66, 30.90, 34.90, 38.04, 45.60, 66.02, 115.18, 127.94, 143.69, 157.22, 173.00, 177.69

Two carboxylic protons were not observed due to the measurement in CD_3OD.

metabolites so far have been rather laborious and inaccurate due to the lack of commercially available authentic standards. The levels of the CAPECs have only been semi-quantified based on the assumption that the response factors of the base ions of these compounds and specific internal standards [1,2,4,5,9], NPEOs and NPECs (nonylphenol ethoxycarboxylic acids) [3,6,7], on gas chromatography–mass spectrometry (GC–MS) or liquid chromatography–mass spectrometry (LC–MS) were the same. Hindered by the lack of authentic standards and effective analytical methods, the literature on the occurrence and behavior of the CAPECs in the environment is still rather scarce compared to the other degradation metabolites such as nonylphenols (NP), short chain NPEOs and NPECs. Consequently, it is of significance to create authentic standards for the CAPECs and then use them for more accurate determination of their concentrations in a typical sample.

In this study, we reported the development of four model CAP1ECs that are characterized by an α,α-dimethyl configuration, five to eight C atoms and a carboxyl group in the alkyl chain, and a carboxymethoxy group (Table 1). The mass spectra of the model compounds from the GC–MS by applying electron ionization (EI) and isobutane chemical ionization (isobutane CI) were extensively interpreted. In addition, the applicability of the GC–EI–MS and GC–isobutane CI–MS on the detection of these model isomers is discussed. To our knowledge, this is the first report interpreting the mass spectra and the detectability of the specific $CA_{5–8}P1ECs$ using GC–EI–MS and GC–isobutane CI–MS based on their corresponding model standards.

2. Experimental

2.1. Materials and reagents

Unless otherwise noted, all reagent grade chemicals and solvents for the synthesis were purchased from Wako (Osaka, Japan), TCI (Tokyo, Japan) and Kanto Chemical (Tokyo, Japan), and used without further purification. *N,O*-Bis(trimethysilyl)acetamide (BSA) and (trimethylsilyl)diazomethane (2 M in hexane) were from Wako and Aldrich (Milwaukee, WI, USA), respectively. The pesticide-grade acetone, methanol and methyl acetate for the preparation of the standard solutions were from Wako and dehydrated by anhydrous sodium sulfate before use. The methyl alcohol-d_4 (CD_3OD) and pyrene-d_{10} were from Isotec (USA) and Kanto Chemical, respectively. The silica gel [BW-127ZH (100–270 mesh)] was from Fuji Silysia (Aichi, Japan).

2.2. Synthesis of dm-$CA_{5–8}$P1ECs

The dm-$CA_{5–8}$P1ECs were obtained through the reaction sequence as shown in Fig. 1. The reactions involved the Friedel–Craft alkylation (a) [11], preparation of

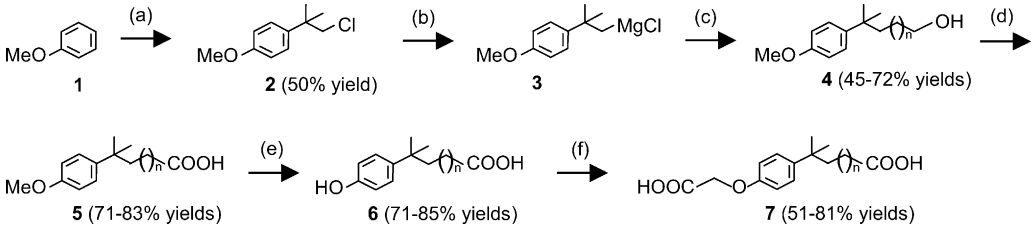


Fig. 1. Reaction sequence for the synthesis of dm-$CA_{5–8}$P1ECs. (a) H_2SO_4 (50 mol%), $CH_2{=}C(CH_3)CH_2Cl$ (0.5 equiv.), 20 °C, 12 h; (b) CH_3I (300 μl), Mg turnings (1.3 equiv.), THF, reflux, 6 h; (c) CuI (0.65 mol%), $Br(CH_2)_{n+1}OH$ (0.4 equiv.), THF, reflux, 4 h; (d) Jones reagent (CrO_3 (2 equiv.) and H_2SO_4/H_2O), acetone, room temperature, 10 h; (e) HBr/AcOH (4 equiv.), 60 °C, 60 h; (f) NaOH (10 equiv.), $ClCH_2COOH$ (5 equiv.), ethanol, reflux, 8 h; n = 1–4.

Grignard reagent (b) [12], cross-coupling (c) [13], oxidation (d) [14], demethylation (e) [15] and carboxymethylation (f) [14]. Briefly, 2-methyl-2-(4-methoxyphenyl)-1-chloropropane (**2**) was formed via the reaction of anisole (2 mol) with 3-chloro-2-methyl-1-propene in the presence of concentrated H_2SO_4. The Grignard reagent **3** prepared from 60 mmol of **2** using magnesium turnings in dehydrated tetrahydrofuran (THF) was separately coupled with the bromoalkanols ($Br(CH_2)_2OH$, $Br(CH_2)_3OH$, $Br(CH_2)_4OH$, or $Br(CH_2)_5OH$) using copper(I) iodide as the catalyst in THF to yield the corresponding alcohols **4**. The alcohols **4** (16 mmol) were then converted into the carboxylic acids **5** using Jones reagent. The carboxylic acids **5** (13 mmol) were subjected to demethylation using HBr/AcOH to yield **6**. The phenolic hydroxyl group of **6** (13 mmol) was then carboxymethylated to yield the target dm-$CA_{5–8}$P1ECs **7**.

The reaction progress was monitored by thin layer chromatography (TLC) and/or GC with flame ionization detection (GC–FID). The products **4**, **5**, **6**, and **7** were purified by column chromatography on a silica gel, while the product **2** was separated from the ortho-byproduct by distillation. The Grignard reagent **3** was immediately used for the coupling reaction. The ^1H and ^{13}C NMR spectra of the dm-$CA_{5–8}$P1ECs (performed on a JEOL JNM-GSX-400 system) are shown in Table 1.

2.3. Standard solution preparation and derivatization

A volume of 100 mg/l stock solutions of the dm-$CA_{5–8}$P1ECs and internal standard (pyrene-d_{10}) were individually prepared in acetone and stored at 4 °C. A working standard mixture of the dm-$CA_{5–8}$P1ECs was prepared by diluting suitable volumes of the individual stock solutions in a 25 ml volumetric bottle with acetone.

BSA and (trimethylsilyl)diazomethane effectively used for derivatization of NPECs [16–20] were separately used in this study in order to augment the correctness of the mass spectra interpretation and select a suitable derivatization method for the determination of the dm-$CA_{5–8}$P1ECs by GC–MS. The derivatization was conducted following reported methods [16,20] with minor modifications. Briefly, after drying a standard solution under a gentle flow of nitrogen gas, the standards were methylated by 50 μl of (trimethylsilyl)diazomethane in 2 ml of methanol containing 1 mg/l internal standard. The trimethylsilylation was conducted by 50 μl of BSA in 0.45 ml of methyl acetate containing 1 mg/l internal standard. Both derivatization reactions were run at 25 °C for 3 h in sealed vials.

2.4. GC–MS analysis of dm-$CA_{5–8}$P1ECs

The analysis of the dm-$CA_{5–8}$P1ECs was conducted on a 3800 gas chromatograph coupled to a Saturn 2000 ion-trap mass spectrometer (Varian, Walnut Creek, CA, USA). A 30 m (0.3 mm i.d. and film thickness = 0.25 μm) fused silica capillary column BPX5 (SGE, Australia) was used. A 2 μl of the methylated standard sample was injected in split/splitless mode (splitless from 1 to 5 min) using programmed temperature vaporization injection while same volume of the trimethylsilylated standard sample was injected in splitless/split mode (splitless from 0 to 1 min) using a normal temperature injection at 280 °C. The injector temperature program was: 70 °C (1 min), 200 °C/min, 280 °C (hold for 8 min). The oven temperature program was: 60 °C (2 min), 14 °C/min, 160 °C, 8 °C/min, 290 °C (hold for 9 min). Helium (99.999%) was used as the carrier gas at a flow rate of 1.2 ml/min.

The EI–MS data were acquired under the following conditions: 100–500 *m/z* mass range, 1 s scan time, 15 min solvent delay, 100 °C manifold temperature, 280 °C transfer line temperature, 200 °C ion trap temperature, 60 μA emission current. In the CI–MS, isobutane was used as the CI reagent gas and the following parameters were applied: 90–500 *m/z* CI-mass range, 2000 μs maximum ionization time, 10 ms maximum reaction time, 35 *m/z* CI-storage level, 30 volts ejection amplitude, and 90 *m/z* background mass.

3. Results and discussion

3.1. Synthesis of dm-$CA_{5–8}$P1ECs

NP in the environment is a mixture of isomers of the nonyl group. Based on the high-resolution GC analysis of

NP, Wheeler et al. recognized that isomers having an α,α-dimethyl structure in the alkyl chain were predominant (accounting for 48.6% of the total para-isomers) [21]. Furthermore, the α,α-dimethyl structure was reported to have a relatively high estrogenic activity among the isomers of NP [22]. Similar to NP, CAP1ECs have been recognized as the metabolites of NPEOs in the environment via oxidation of the ethoxy and alkyl side chains [7,10]. Therefore, CAP1ECs are expected to encompass isomeric mixtures of branched carboxyalkyl structures that are impossible to presently prepare in the laboratory. The information on the predominance and estrogenicity of the α,α-dimethyl structure of NP prompted us to synthesize this structural type of CAP1ECs to aid in the more accurate determination of these metabolites in the environment.

Because of their similar structures (only the number of carbon atoms in the alkyl chain varied), the four model CAP1ECs were effectively synthesized by the same reaction sequence as shown in Fig. 1. The reaction of the anisole with 3-chloro-2-methyl-1-propene in concentrated H_2SO_4 yielded the corresponding *o*- and *p*-products mixture. The distillation of the mixture yielded 50% of **2**. The respective coupling reaction of the Grignard reagent **3** with bromoalkanols in refluxing THF yielded the products **4** that contain hydroxyl group in the alkyl chain in 45–72% yields (based on bromoalkanol). The oxidation of the hydroxyl group of **4** by Jones reagent gave the carboxylic acids **5** in 71–81% yields. Similarly, the products **6**, which were formed by the demethylation of the methoxy group with HBr/AcOH were obtained in 71–85% yields. The carboxymethylation of **6** with $ClCH_2COOH$ in refluxing ethanol gave the target products **7** in 51–81% yields.

3.2. EI-mass spectra of dm-$CA_{5–8}$P1ECs

Fig. 2a and b, respectively, show EI-mass chromatograms of the methyl and trimethylsilyl dm-$CA_{5–8}$P1ECs upon analyzing 200 μg/l standard solutions by GC–EI–MS. In general, dm-$CA_{5–8}$P1ECs were separated by approximately 1 min, starting with dm-CA_5P1EC. The methyl derivatives eluted from the GC system faster than the corresponding trimethylsilyl derivatives under the same GC conditions.

For all the trimethylsilyl and methyl derivatives, the most significant ions were at $m/z = 265$ and 207, respectively, corresponding to the α,α-dimethyl structures via the benzylic cleavage of the carboxyalkyl chain (Fig. 2). The other fragment ions showed very low intensities (less than 5% abundance). The fragmentation pathway of the dm-$CA_{5–8}$P1ECs was similar to those reported for the metabolites such as NP, NPEOs and NPECs [20,21]. The mass spectra interpretation of the dm-$CA_{5–8}$P1ECs agreed well with those of the CAPEC metabolites mainly extracted from the different environmental samples in previous reports [1,2,4,5,9].

As already mentioned, all the trimethylsilyl dm-$CA_{5–8}$P1ECs afforded the same base ion peaks of $m/z = 265$ similar to the trimethylsilyl NP1EC [20]. It is unlikely that NP1EC will impact the quantification of the dm-$CA_{5–8}$P1ECs as the NP1EC derivatives elute more quickly than the dm-$CA_{5–8}$P1ECs (data not shown).

3.3. Isobutane CI-mass spectra of dm-$CA_{5–8}$P1ECs

Table 2 shows the major fragment ions and their abundances for the methyl and trimethylsilyl dm-$CA_{5–8}$P1ECs upon analyzing 500 μg/l standard solutions by isobutane CI–MS. Interestingly, the abundances of the ions resulting from the same derivatization and fragmentation were not united except for **D** (100% abundance) in the trimethylsilylation and **B** (100% abundance) in the methylation although the only structural difference was the length of the alkyl chain. From the resulting fragment ions, the fragmentation pathway of the dm-$CA_{5–8}$P1ECs was proposed as shown in Fig. 3. The fragmentation included a proton transfer chemical ionization and simultaneously occurring electron ionization.

In general, in CI–MS, a relatively stable $[M+H]^+$ ion is formed via the proton transfer from the reagent ion to the analyte. Previous studies reported the relatively high abundances of $[M+H]^+$ ions of the CAPECs when

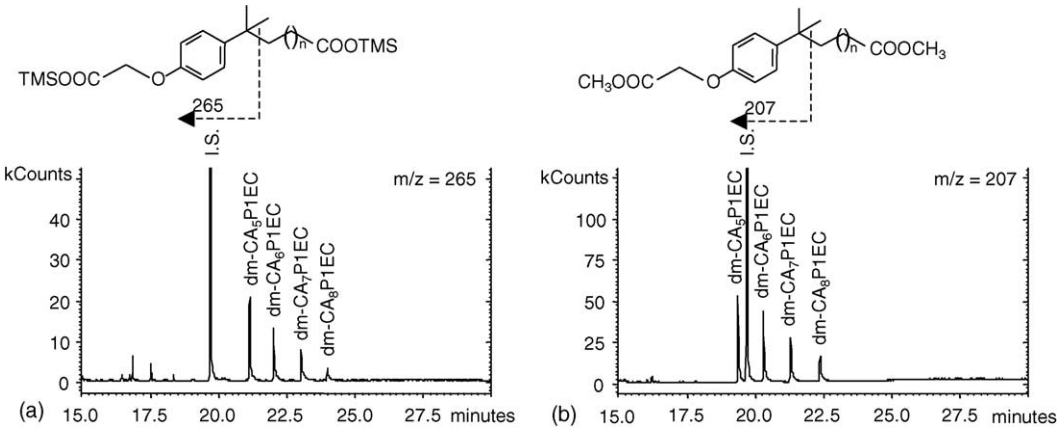


Fig. 2. EI-mass chromatograms of (a) trimethylsilyl and (b) methyl dm-$CA_{5–8}$P1ECs.

Table 2
Major fragment ions and their relative abundances of dm-$CA_{5–8}$P1ECs in CI–MS

CAP1EC	Trimethylsilyl derivative								Methyl derivative							
	MW	A	B	C	D	E	F	G	MW	A	B	C	D	E	F	G
dm-CA_5P1EC	410		187 (45)	97 (5)	321 (100)		410 (5)	265 (25)	294		129 (100)	97 (5)	263 (68)		294 (7)	207 (20)
dm-CA_6P1EC	424	425 (4)	201 (78)	111 (5)	335 (100)			265 (31)	308		143 (100)	111 (8)	277 (8)		308 (4)	207 (19)
dm-CA_7P1EC	438	439 (3)	215 (65)	125 (14)	349 (100)	291 (43)		265 (29)	322		157 (100)	125 (14)	291 (11)	233 (7)		207 (22)
dm-CA_8P1EC	452	453 (3)	229 (9)	139 (6)	363 (100)	305 (22)		265 (28)	336		171 (100)	139 (17)	305 (89)	247 (23)		207 (30)

All entries are the *m/z* values. The relative abundance is given in parentheses.

analyzing the environmental samples by methane CI–MS [1,2,4]. However, the isobutane CI-mass spectra of the derivatives of the dm-$CA_{5–8}$P1ECs, given in Table 2, were not consistent with the generalization. The $[M+H]^+$ ions, **A**, were understood to be mostly decomposed by undergoing extensive fragmentation to give many fragment ions since they were not completely observed (for methyl dm-$CA_{5–8}$P1ECs) or observable at a very low intensity (for trimethylsilyl dm-$CA_{5–8}$P1ECs). In addition to the very low signals of the molecular ions, **F**, the fragment ions, **G**, at $m/z = 207$ and 265 (corresponding to the benzylic cleavage) of the respective methyl and trimethylsilyl dm-$CA_{5–8}$P1ECs were also detected although less prominently, suggesting that EI and CI occurred simultaneously in the ion trap.

The methyl dm-$CA_{5–8}$P1ECs afforded base peak ions, **B**, at $m/z = 129$, 143, 157, and 171, corresponding to the loss of the methyl phenoxyacetate from the dm-CA_5-, dm-CA_6-, dm-CA_7- and dm-CA_8P1EC, respectively. Meanwhile, the most significant fragment ions, **D**, of the trimethylsilyl dm-$CA_{5–8}$P1ECs were at $m/z = 321$, 335, 349, 363 corresponding to the loss of the trimethylsilanol from **A**.

The moderate and strong fragment ions, **D**, should be formed by loss of ROH from the alkyl chain since these ions were not observed with the derivatives of NP1EC (data not shown). The loss of ROH from the phenoxycarboxylate side of both the methyl and trimethylsilyl dm-$CA_{5–8}$P1ECs were not observed. However, relatively intense fragment ions, **E**, at $m/z = 233$ and 291, or at $m/z = 247$, 305 were, respectively, observed in the mass spectra of the methyl and trimethylsilyl dm-CA_7P1ECs, or the methyl and trimethylsilyl dm-CA_8P1ECs. By losing 58 amu from ion **D**, these fragment ions could result from the loss of a three-membered lactone via the methyl and trimethylsilyl rearrangements ($[\mathbf{D} - C_2H_2O_2]^+$) [20] and used as the characteristic fragment ions of dm-CA_7P1EC and dm-CA_8P1EC.

The fragment ions, **C**, at $m/z = 97$, 111, 125, 139 could result from the simultaneous loss of methanol and methyl phenoxyacetate from the methyl dm-$CA_{5–8}$P1ECs, or of trimethylsilanol and trimethylsilyl phenoxyacetate from the trimethylsilyl dm-$CA_{5–8}$P1ECs.

Our results confirmed the fragmentation pathway interpreted by Ding and Tzing [2] except for the formation of ions **E**.

3.4. Detectability of dm-$CA_{5–8}$P1ECs by GC–EI–MS and GC–isobutane CI–MS

The fragment ions for the identification and quantification of the methyl and trimethylsilyl dm-$CA_{5–8}$P1ECs as well as the sensitivities of these compounds when analyzed by EI–MS and isobutane CI–MS are given in Table 3. The highest intensity and the most informative ions were selected so that the characterizing ions and the possibly highest intensities of the compounds were taken into account. The sensitivities of the dm-$CA_{5–8}$P1ECs were evaluated by instrumental detection limits. To our knowledge, this is the

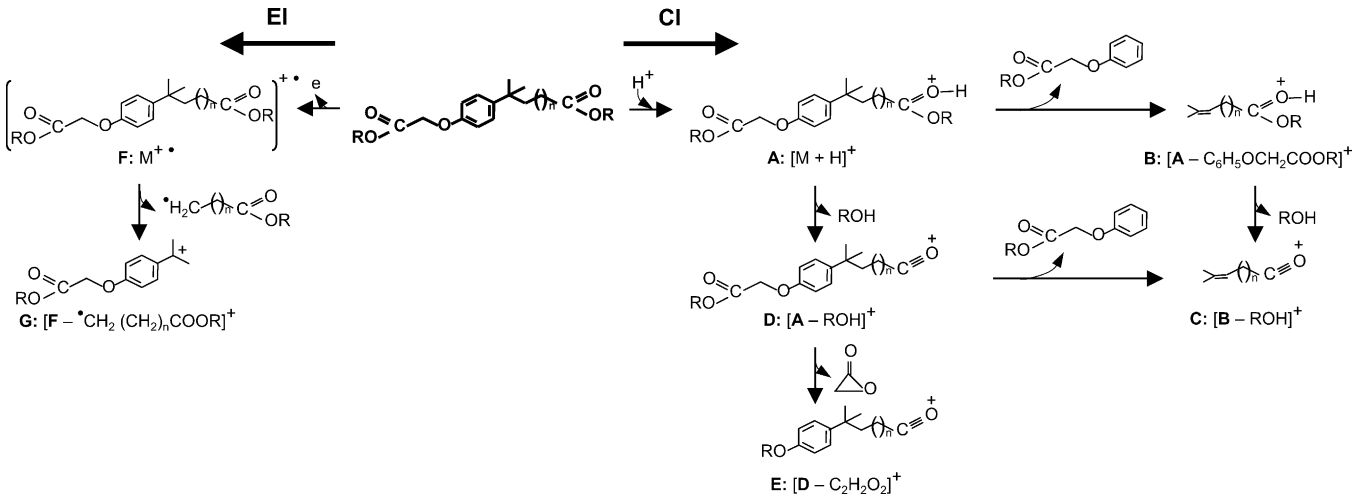


Fig. 3. General scheme for the fragmentation of dm-$CA_{5–8}$P1ECs in CI–MS (n = 1–4; R = methyl or trimethylsilyl).

Table 3
Selected parameters for the determination of dm-$CA_{5–8}$P1ECs derivatives and detection limits

Derivatization	Ionization		dm-CA_5P1EC	dm-CA_6P1EC	dm-CA_7P1EC	dm-CA_8P1EC
Methylation	EI	Retention time (min)	19.38	20.30	21.30	22.37
		Confirmation ion (*m*/*z*)	207	207	207	207
		Quantification ion (*m*/*z*)	207	207	207	207
		IDL[a] (pg)	5.4 (1.7)	9.6 (3.1)	17 (2.7)	22 (3.5)
	CI	Confirmation ion (*m*/*z*)	129, 207, 263	143, 207, 277	157, 207, 291	171, 207, 305
		Quantification ion (*m*/*z*)	129 + 263	143 + 277	157 + 291	171 + 305
		IDL[a] (pg)	9.4 (3.0)	21 (6.8)	34 (5.5)	34 (5.4)
Trimethylsilylation	EI	Retention time (min)	21.16	22.02	23.03	23.97
		Confirmation ion (*m*/*z*)	265	265	265	265
		Quantification ion (*m*/*z*)	265	265	265	265
		IDL[a] (pg)	25 (8.0)	28 (9.0)	38 (6.1)	45 (7.1)
	CI	Confirmation ion (*m*/*z*)	187, 265, 321	201, 265, 335	215, 265, 349	229, 265, 363
		Quantification ion (*m*/*z*)	187 + 321	201 + 335	215 + 349	229 + 363
		IDL[a] (pg)	27 (8.5)	32 (10)	45 (7.1)	60 (9.6)

The relative standard deviation is given in parentheses. The quantification was carried out by the internal standard method using the response factors of the selected fragment ions to the molecular ion of internal standard (*m*/*z* 212 in EI and *m*/*z* 213 in CI).

[a] Instrumental detection limit: calculated from the mean and standard deviation estimates with $n - 1$ degrees of freedom and 97% confidence level of seven replicates of the 50 μg/l working standard (except for dm-CA_7P1EC and dm-CA_8P1EC = 100 μg/l).

first time the detection limits of specific CAP1ECs isomers have been reported.

In general, the detection limits of the methyl dm-$CA_{5–8}$P1ECs, which ranged from 5.4 to 22 pg in EI and 9.4–34 pg in CI, were relatively lower than those of the trimethylsilyl dm-$CA_{5–8}$P1ECs, which ranged from 25 to 45 pg in EI and 27–60 pg in CI. In addition, the detection limits of the methyl and trimethylsilyl dm-$CA_{5–8}$P1ECs in CI were, respectively, 1.5–2.2 and 1.1–1.3 times higher than those in EI.

By applying GC–EI–MS, we could identify and quantify the total amount of the mixture of CA_5-, CA_6-, CA_7-, and CA_8P1EC metabolites using synthetic dm-$CA_{5–8}$P1ECs (based on base peak ion at m/z = 207 for the methylation or m/z = 265 for the trimethylsilylation) instead of an internal standard, NPEOs or NPECs. However, because of having the same base peak ions, the individual isomeric mixtures of the $CA_{5–8}$P1ECs metabolites could not be distinguished unless they were clearly separated by the GC system.

Despite having the higher detection limits, we realized that GC–isobutane CI–MS would be an adequate method because the dm-$CA_{5–8}$P1ECs could be present at high levels in environmental samples ($CA_{5–8}$P1ECs were estimated to be at high, microgram per liter, levels [1–6,9]). In addition, by applying GC–isobutane CI–MS, the individual branched carboxyalkyl isomer mixtures of the $CA_{5–8}$P1EC metabolites could be selectively identified as they are expected to give the same characterizing fragment ions with the corresponding dm-$CA_{5–8}$P1ECs. The individual branched carboxyalkyl isomeric mixtures of the adjacent metabolites

could be distinguished by the difference of 14 amu in their characterizing ions even if they are unable to be completely separated by the GC system. The quantification of $CA_{5–8}$P1ECs could also be more accurate when they were calculated based on the corresponding dm-$CA_{5–8}$P1ECs than the previously reported values [1–7,9].

4. Conclusions

The synthesis of four model CAP1ECs having an α,α-dimethyl structure in their carboxyalkyl chains was effectively demonstrated. By interpreting the mass spectra obtained from the GC–EI–MS and GC–isobutane CI–MS, we realize that the methyl or trimethylsilyl $CA_{5–8}$P1ECs metabolites could be more accurately determined using synthetic dm-$CA_{5–8}$P1ECs in the CI–MS mode although the detection limits of these isomers were observed to be lower in the EI–MS mode. Further research on the development of a routine analytical method to evaluate the occurrence of $CA_{5–8}$P1ECs in water and sediment of receiving water and the effluents of a wastewater treatment plant is now in progress.

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