Journal of Chromatography B, 900 (2012) 64–70

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Development of a three-steps derivatization assay for the localization of double bond in monounsaturated monomers of poly-beta-hydroxyalkanoates by GC–MS

Christelle Simon-Colin[a,*], Christelle Gouin[a], Pierre Lemechko[a,b], Nelly Kervarec[c], Jean Guezennec[a]

[a] Institut Français de Recherche pour l'Exploitation de la Mer, Centre de Brest, RBE/BRM/LBMM, B.P. 70, 29280 Plouzané, France
[b] Institut de Chimie et des Matériaux de Paris Est (ICMPE) UMR 7182, Université Paris Est, 2 à 8 rue Henri Dunant, 94320 Thiais, France
[c] Service Commun de RMN-RPE, UBO, 6 Avenue Le Gorgeu, 29200 Brest, France

ARTICLE INFO

Article history:
Received 12 March 2012
Accepted 25 May 2012
Available online 2 June 2012

Keywords:
Poly-beta-hydroxyalkanoate
Unsaturation
DMDS derivatives
Acetylation
GC–MS

ABSTRACT

A new gas chromatography–mass spectrometry (GC–MS) method for the localization of double bond in monounsaturated 3-hydroxyalkenoic acids monomers has been developed. A three steps derivation assay was used including a methanolysis, then acetylation and dimethyldisulfide (DMDS) addition to alkene groups. Electron impact GC–MS analysis of such derivatives offers characteristic fragments allowing the unambiguous determination of double bond position in side chain. This novel method is well-suited for the routine analysis of poly-beta-hydroxyalkanoates (PHAs), and was used to characterize monounsaturated monomers in both 3-hydroxyalkenoic acids standards as well as in mcl-PHAs and poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate) (PHOU) produced by bacterial strain *Pseudomonas guezennei* from glucose or a mixture of sodium octanoate plus 10-undecenoic acid, respectively.

1. Introduction

Poly-beta-hydroxyalkanoates (PHAs) are biopolymers stored in intracellular inclusion bodies by a wide variety of bacteria as an energy reserve, in response to excess carbon under nutrient-limited conditions [1,2]. The monomeric composition of PHAs can be related to the nature of the carbon source supplied to the bacteria. PHAs exhibit a great variety of properties and thus may have different applications. Regarding the length of their carbon chains, PHAs can be divided in three groups: short chain length PHAs (3–5 carbon atoms, scl-PHAs), medium chain length (6–15 carbon atoms, mcl-PHAs) and long chain length (more than 15 carbon atoms, lcl-PHAs). Due to structural differences, the physical properties of mcl-PHAs are generally quite different from scl-PHAs such as poly(3-hydroxybutyrate) (PHB) that is highly crystalline and stiff material whereas mcl-PHAs have elastomeric properties. Owing to their inherent biocompatibility and biodegradability, PHAs have attracted industrial interest and have been extensively studied in the last two decades [3–6]. They are regarded as promising substitutes for petrochemicals plastics, and thus for use in tackling the problem of plastic waste in the future. Moreover, PHAs can be produced from natural renewable carbon sources and represent a new way of utilizing waste from low cost carbon stocks [7–11]. In particular, mcl-PHAs show great promise as thermoelastomers for biomedical applications [12], such as drug delivery [13] and tissue engineering [14,15]. Actually PHAs containing long chain saturated and unsaturated monomers have unique properties, and double bonds hold the possibility for further chemical modifications. New functional groups such as epoxide [16], carboxylic acid [17,18], chlorine [19], hydroxyl groups [20–22] and alkyne groups ([23] can be used to further conjugate oligomers, bioactive compounds or targeting molecules. Moreover, while many different PHAs have been reported, relatively few have been produced in useful quantities and fewer still meet properties necessary to replace synthetic polymers. Therefore, modification of natural PHAs is often necessary to impart properties at least equivalent to their synthetic counterparts and make PHAs viable replacement candidates.

One prerequisite for successful functionalization is a good knowledge of the chemical composition of PHAs in particular carbon chain length of the different monomers, number of olefinic groups, and location of double bonds. There are several analytical and spectrometric methods that can be used for the analysis of PHAs. Nuclear magnetic resonance (NMR) spectrometry is a very useful technique for the characterization of PHAs, especially for scl-PHAs whose signals are easily attributed to the different atoms of the side chains on one-dimensional proton and carbon spectra. However the structural elucidation of PHAs containing saturated and unsaturated monomers possessing long chain length requires two-dimensional homonuclear and heteronuclear techniques that are time-consuming measurements, and not always obvious [24–27].

The need for faster analytical methods for the determination of PHAs forced the development of chromatographic methods such

* Corresponding author. Tel.: +33 02 98 22 45 28; fax: +33 02 98 22 47 57.
E-mail address: christelle.simon.colin@ifremer.fr (C. Simon-Colin).

1570-0232/$ – see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.jchromb.2012.05.032

as the powerful separation technique of gas chromatography (GC). This requires a quantitative depolymerization of the polymer, usually combined with a derivation. The first derivation methods for GC were developed for PHB. The method developed by Braunegg et al. [28] consisting in acidic extraction, hydrolysis and methylation using 3% H_2SO_4, was a major advance for the analysis of PHAs because, in contrast to the alkaline hydrolysis used by Wallen and Rohwedder [29] which inevitably led to a mixture of 3-hydroxy acid methyl esters and 2-alkenoic acid methyl esters, the mild acidic hydrolysis resulted only in the formation of 3-hydroxy acid methyl esters. Langeveen et al. [30] expanded the method described by Braunegg et al. [28] to mcl-PHAs by using 15% H_2SO_4 as the transmethylation reagent in methanol. Huijberts et al. [31] found that the hydrolysis time for mcl-PHAs should be increased to 4 h, and demonstrated that no degradation of methyl esters occurred 24 h after hydrolysis. Findlay and White [32], Riis and Mai [33], and Bear et al. [16] preferred to use HCl to hydrolyze PHAs, due to further decomposition of the 3-hydroxy esters in presence of sulfuric acid by acid catalyzed elimination. More recently, Furrer et al. [27] recommended the use of boron trifluoride in methanol to prevent the formation of side-products during the transesterification of olefinic mcl-PHAs.

The electron impact gas chromatography mass spectrometry (GC–MS) analysis of 3-hydroxyalkanoic acids methyl esters gives a characteristic fragment at *m*/*z* 103 resulting from the cleavage α to the hydroxyl functional group and the fragment *m*/*z* 74 due to the McLafferty rearrangement. However, this analysis without any further chemical derivatization provides little structural information on the chain length. Some appropriate chemical derivatizations of 3-hydroxyalkanoic acids methyl esters as trimethylsilylation (TMSi) are useful for the structural characterization of PHAs monomers [34]; TMSi derivatives of 3-hydroxyalkanoic acids methyl esters provide very distinct molecular ion-related fragments and clearly reflects their chain length, as well as the unsaturation degree. However, even though Lee and Choi [34] observed some changes in fragmentation pattern of TMSi derivatives of 3-hydroxylalkanoic methyl ester of saturated and unsaturated monomers in relation with the double bond position, there is still no chromatographic method that enables unambiguously the localization of double bond whatever its position and monomer side chain length.

In this study, we developed a novel and efficient method by gas chromatography mass spectrometry GC–MS for the localization of double bond in monounsaturated 3-hydroxyacids monomers units of mcl-PHAs. The method was based on a two steps process. First, TMSi methyl esters derivatives were prepared to access to monomers side chain length as well as the number of double bonds. In a second step, preparation of thiomethylated acetylated methyl esters derivatives using three successive derivations including methanolic hydrolysis, acetylation and thiomethylation by iodine-catalyzed dimethylsulfide (DMDS) addition to double bond, was performed for the localization of olefin groups. The electron ionization GC–MS analysis of such derivatives leads to characteristic mass spectra fragments that enable the unambiguous determination of the double bond position.

2. Experimental

2.1. Standards of 3-hydroxyalkenoic acids

Standards of 3-hydroxyoct-5-enoate (3HO:1Δ5), 3-hydroxynon-6-enoate (3HN:1Δ6), 3-hydroxydec-7-enoate (3HD:1Δ7), 3-hydroxyundec-8-enoate (3HUD:1Δ8), 3-hydroxydodec-6-enoate (3HDD:1Δ6), 3-hydroxydodec-9-enoate (3HDD:1Δ9), were purchased from Epsilon Chimie (Brest, France).

2.2. Preparation of derivatives for GC–MS

Both the length and number of double bonds of the 3-hydroxyalkanoic monomers were determined by GC–MS analysis of the TMSi derivatives of methyl esters. A 7 mg PHAs sample or 2 mg for 3-hydroxyalkenoic acids standards was dissolved in 1.5 mL chloroform and subjected to methanolysis in the presence of MeOH–HCl 37% (17:2, v/v) at 100 °C for 4 h (for polymer) or 1 h (for standards), in Pyrex test tubes (volume 10 mL) with screw Teflon-lined caps. After phase separation and two washes with 1 mL distilled water, the organic phase was dried with $MgSO_4$ and evaporated under nitrogen. TMSi derivation of 3-hydroxyalkanoates methyl esters was accomplished by adding 100 μL pyridine and 100 μL sylon (BSTFA–TMCS, 99:1) to methanolized sample. The reaction mixture was heated at 70 °C for 45 min.

The localization of olefin groups on monounsaturated chains was performed using a three derivations process: methanolysis, acetylation onto the 3-hydroxyl group, and thiomethylation by grafting a thiomethyl group on each side of the double bond. Samples of polymer and 3-hydroxyalkenoic acids standards were subjected to methanolysis in the same conditions as above. The methyl esters were then acetylated in the presence of 100 μL acetyl chloride at 65 °C for 5 min to mask the reactive hydroxyl group, as described by Johnson and Trinh [35]. The mixture was evaporated to dryness with nitrogen. Hexane (500 μL), dimethyldisulfide DMDS (100 μL) and iodine I_2 (20 μL) were added, the mixture heated at 50 °C for 48 h as described elsewhere [36,37]. Samples were diluted with 200 μL of hexane, and the iodine in excess was reduced by addition of Na_2SO_3. After centrifugation, thiomethyl acetylated methyl esters derivatives were recovered from the supernatant, dried under nitrogen and dissolved in 500 μL dichloromethane prior to analysis by GC–MS.

2.3. GC–MS conditions

The TMSi methyl esters derivatives and thiomethyl acetylated methyl esters derivatives were analyzed by GC–MS using an Agilent 6890N chromatograph coupled to a quadrupole Agilent 5975 inert XL mass selective spectrometer, equipped with a HP-5-MS fused silica capillary column (30 m × 0.25 mm, 25 μm film thickness). A 1 μL sample was injected (spit ratio 100:1) with helium as carrier gas and the temperature was programmed for the separation of peaks (60 °C for one min, ramp of 4 °C/min to 140 °C, 15 °C/min to 280 °C and 5 min at 280 °C). The ionizing energy for MS operation was 70 eV.

2.4. NMR analyses

NMR spectroscopy was performed at 25 °C on samples of PHAs dissolved in $CDCL_3$ on a BRUKER 400 DRX spectrometer equipped with a 5 mm triple resonances $^1H/\{BB\}/^{31}P$ (Bruker, Germany) operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm relative to signal of 3,3,3,4-tetramethyl silane.

2.5. Biosynthesis of mcl-PHAs

Pseudomonas guezennei (strain CNCM-I-3358 in the Collection Nationale de Cultures de Microorganismes, Institut Pasteur, Paris, France) was cultivated in a two-steps batch cultivation process. In the first step, the cells were inoculated at 10% (v/v) with a suspension of cells in exponential phase and grown in 5 L fermenter (Infors, Massy, France) containing 3 L of rich marine broth medium (10 g peptone, 5 g yeast, 15 g sea salts per liter distilled water). The temperature was maintained at 35 °C and the pH was adjusted at 7.0 by automatic addition of 2 M NaOH. The air flow was fixed at 30 L/h and the agitation rate from 200 to 800 rpm to

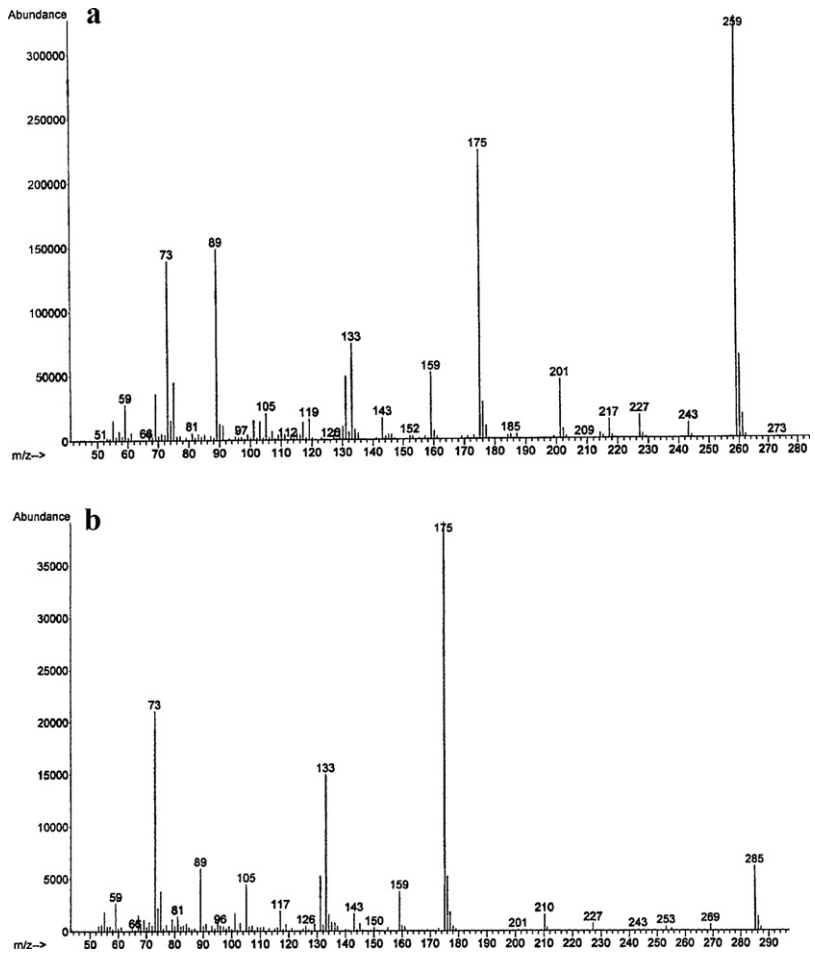

Fig. 1. Mass spectra of the TMSi derivatives of 3-hydroxydecanoic acid (3HD) (a) and 3-hydroxydodecenoic acid (3HDDe) (b) methyl esters. The characteristic peaks and molecular ion-related fragments are assigned as described in the text.

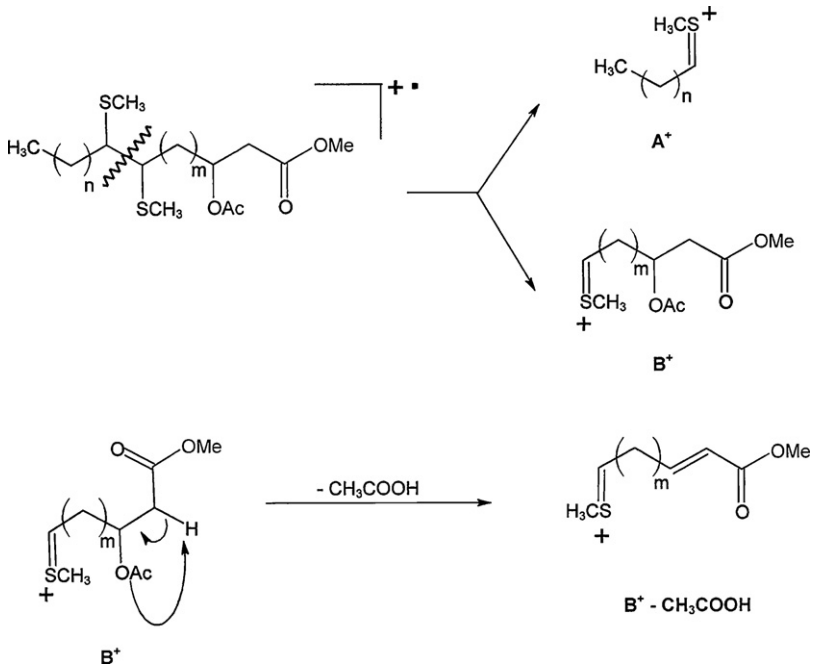

Fig. 2. Mass fragmentation scheme of thiomethyl acetylated methyl esters derivatives of 3-hydroxyalkenoic acids.

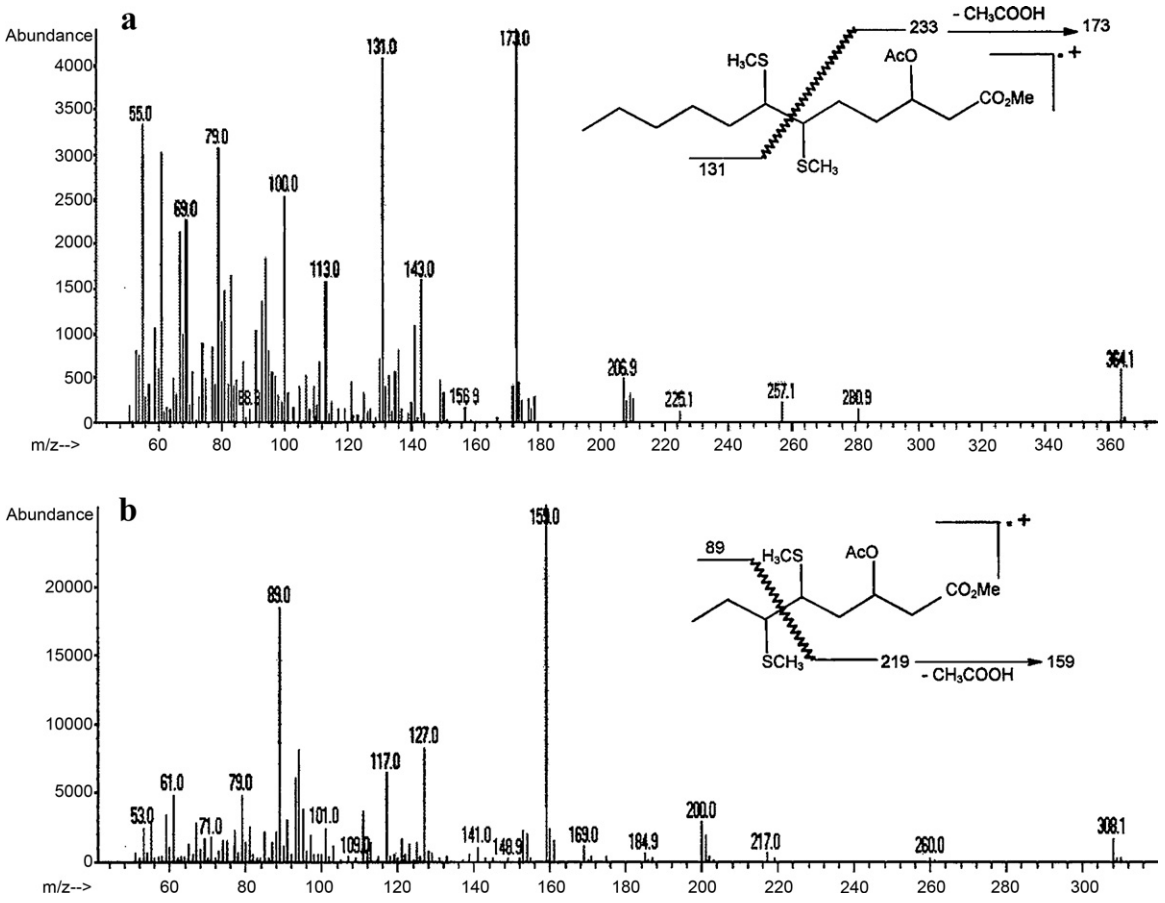

Fig. 3. Mass spectra of thiomethyl acetylated methyl esters derivatives of 3-hydroxydodec-6-enoic acid (3HDD:1Δ6) ([M] = 364) (a) and 3-hydroxyoct-5-enoic acid (3HO:1Δ5) ([M] = 308) standards.

maintain the level of dissolved O_2 at its maximum. After the cultivation for 8 h, cells were harvested by centrifugation (5000 × *g*, 20 min), and transferred into 5 L fermenter containing 3 L nitrogen-free medium (15 g/L sea salts) enriched with 20 g/L of glucose or, a mixture of 3 g/L sodium octanoate and 10-undecenoic acid with concentration varying from 0.2 to 1 g/L. Culture was incubated at 35 °C, 200–400 rpm, and dissolved O_2 maintained around 25%. Following cultivation for 60 h, cells were harvested by centrifugation (10,000 × *g* for 15 min), washed-up three times with diluted seawater, and the pellets lyophilized prior to PHAs extraction.

Pellets of freeze-dried cells were ground with a mortar and pestle, the resulting powder was extracted with chloroform for 4 h at 50 °C. The PHAs-containing chloroform phase was washed once with water to remove residual solid particles, and concentrated. The organic phase was evaporated to dryness, and purified PHAs were obtained by repeated precipitations in 10 volumes of cold methanol.

3. Results and discussion

3.1. Determination of PHAs monomers chain length and unsaturation degree

GC–MS analyses of TMSi methyl esters derivatives of PHAs allowed us to determine the monomeric composition of PHAs obtained from *P. guezennei*. The mass spectra of TMSi derivatives of 3-hydroxyalkanoic acids exhibited characteristic fragments [$(CH_3)_3SiO^+$=$CHCH_2CO_2CH_3$] at *m*/*z* 175 and [RCH=$O^+Si(CH_3)_3$]

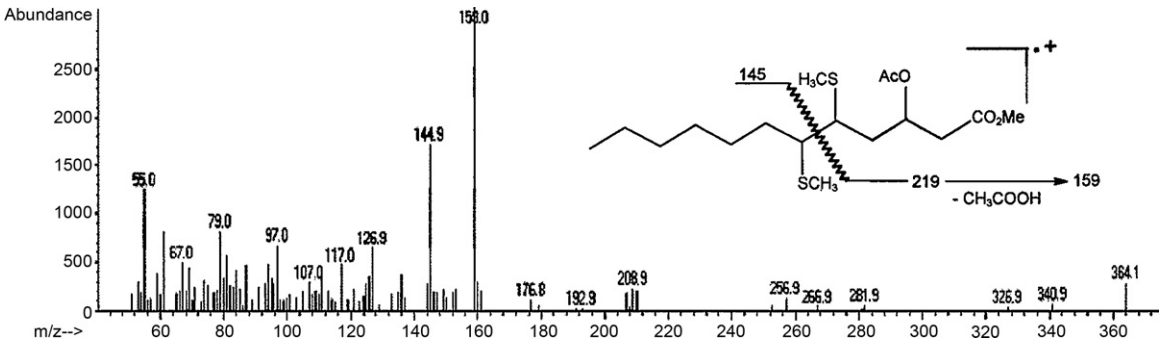

Fig. 4. Mass spectra of thiomethyl acetylated methyl esters derivative of 3-hydroxydodec-5-enoic acid (3HDD:1Δ5) ([M] = 364) as a constituting monomer of mcl-PHAs produced by *P. guezennei* cultivated on glucose.

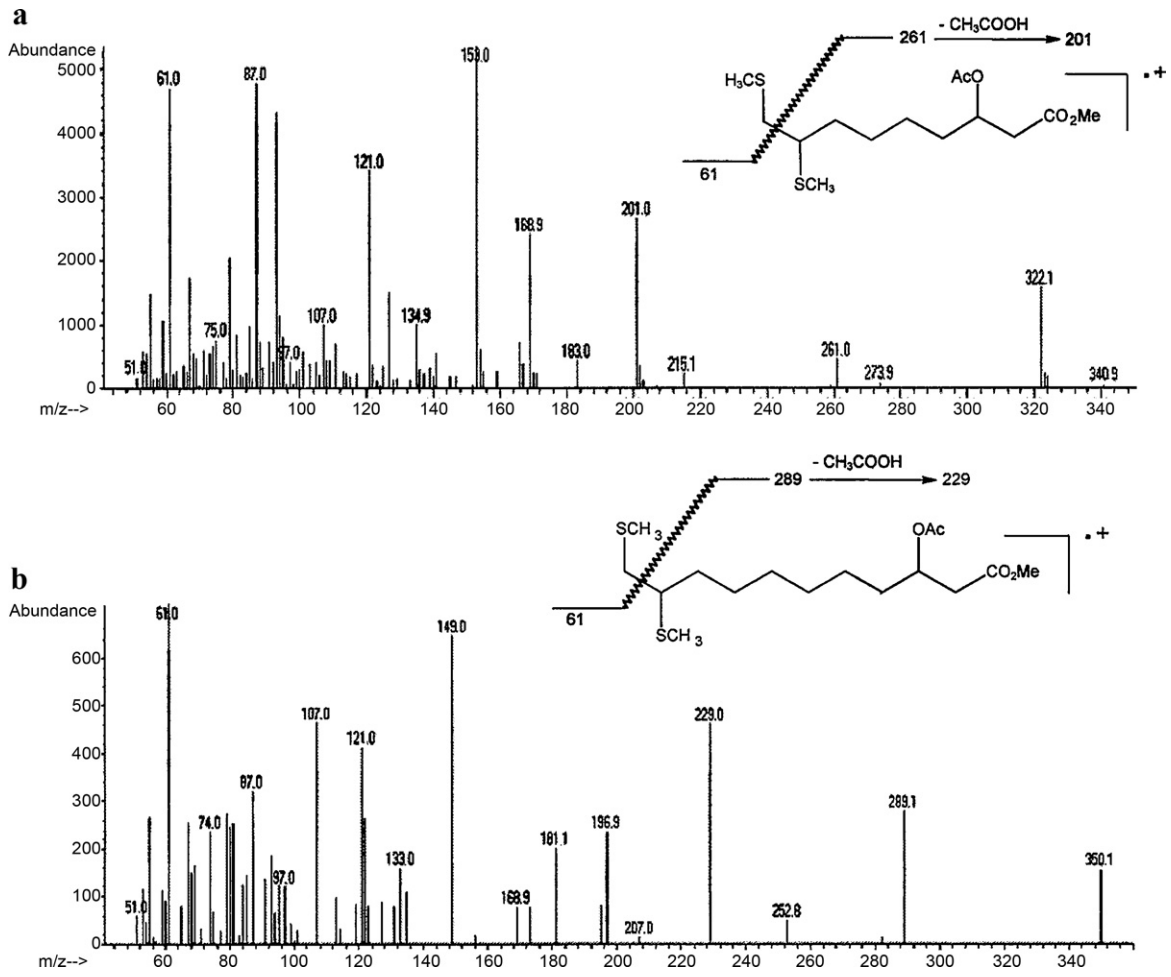

Fig. 5. Mass spectra of thiomethyl acetylated methyl esters derivatives of 3-hydroxynon-8-enoic acid (3HN:1Δ8) ([M]=322) (a) and 3-hydroxyundec-10-enoic acid (3HUD:1Δ10) ([M]=350) (b) as constituting monomers of PHOU produced by *P. guezennei* from sodium octanoate plus 10-undecenoic acid.

Table 1
Monomeric composition of mcl-PHAs produced by *P. guezennei* from glucose and a mixture of sodium octanoate (Oct) plus 10-undecenoic acid (Und).

	PHAs monomeric composition mol%								
Carbon source	3HHx	3HHp	3HHp:1	3HO	3HN:1	3HD	3HUD:1	3HDD	3HDD:1
Glucose 20 g/L	1			24		66		7	2
Oct 3 g/L + Und 1 g/L	1.8		1	72	18	1.4	5.8		
Oct 3 g/L + Und 0.5 g/L	1		0.9	78	13	2	4.6		
Oct 3 g/L + Und 0.21 g/L	2.1		0.8	84	6	4.7	2.3		

at *m*/*z* [$M^{\bullet+}$−73] resulting from α-cleavage of the derivatized hydroxyl group (Fig. 1). The TMSi ethers do not usually show a parent molecular ion but the molecular ion related fragment at *m*/*z* [$M^{\bullet+}$−15] is quite prominent and can be used to determine the chain length of monomers. The existence of a double bond can be deduced from the molecular weight of the fragment at *m*/*z* [$M^{\bullet+}$−15] which is two amu less than that of the corresponding saturated monomer, and from the shift of the base peak as already

Table 2
GC–MS fragmentation pattern of thiomethyl acetylated methyl esters derivatives of monounsaturated 3-hydroxyalkenoic acids issued from standards or mcl-PHAs produced by *P. guezennei*. Putative values of *m*/*z* for fragment [B^+] are indicated between parentheses when it is not detected.

	[$M^{\bullet+}$]	[A^+]	[B^+]	[B^+—HOAc]
3HHp:1Δ6	294	61	(233)	173
3HO:1Δ5	308	89	(219)	159
3HN:1Δ6	322	89	(233)	173
3HN:1Δ8	322	61	261	201
3HD:1Δ7	336	89	247	187
3HUD:1Δ8	350	89	261	201
3HUD:1Δ10	350	61	289	229
3HDD:1Δ5	364	145	(219)	159
3HDD:1Δ6	364	131	233	173
3HDD:1Δ9	364	89	275	215

described by Lee and Choi [34]. The authors also observed changes in the intensity ratio of the related ion peak (at m/z [$M^{\bullet+}-15$]) to the base peak depending on the position of the double bond ($\Delta 5$ or $\Delta 7$).

Others fragments of lower mass were also common for the 3-hydroxyl functional group and readily assignable: m/z 73 [$(CH_3)_3Si^+$], m/z 89 [$(CH_3)_3SiO^+$], m/z 131 $(C_5H_{11}SiO_2)^+$, m/z 159 $(C_6H_{11}SiO_3)^+$ and m/z 133 $(C_5H_{13}SiO_2)^+$.

The composition of PHAs synthesized by *P. guezennei* from glucose was found to mainly consist of 3-hydroxydecanoate (3HD) and 3-hydroxyoctanoate (3HO), and low fractions of 3-hydroxydodecanoate (3HDD), 3-hydroxydodecenoate (3HDDe) and 3-hydroxyhexanoate (3HHx), as identified by GC–MS (Table 1). The presence of olefinic groups along the alkyl side chain, usually characterized by additional signals around 5.3, 2 and 2.3 ppm in the ^{1}H NMR spectrum and signals between 120 ppm and 135 ppm in the ^{13}C NMR spectrum (data not shown), was not detected by NMR because of the low amount (2%) of the unsaturated monomers.

Conversely, the use of sodium octanoate in mixture with 10-undecenoic acid as carbon sources for the production of PHAs by *P. guezennei* lead to the formation of a mcl-PHAs with more unsaturated monomers. Six different TMSi methyl esters peaks were found: 3-hydroxhexanoate (3HHx), 3-hydroxyheptenoate (3HHp:1), 3-hydroxyoctanoate (3HO), 3-hydroxynonenoate (3HN:1), 3-hydroxydecanoate (3HD) and 3-hydroxyundecenoate (3HUD:1), present in different proportions depending on the composition of the culture medium. mcl-PHAs produced by *P. guezennei* from sodium octanoate plus 10-undecenoic acid are mainly composed by 3HO together with 3HN:1 and 3HUD:1, that characterized PHOU (poly(3-hydroxyoctanoate-*co*-3-hydroxyundecenoate). The alkene groups ratio was directly controlled by the 10-undecenoic acid concentration in the culture medium with values ranging from 9.1% unsaturated pendant groups with 0.2 g/L 10-undecenoic acid, to 18.5% and 24.8% with 0.5 and 1 g/L 10-undecenoic acid, respectively. Highly unsaturated PHOU with 43% unsaturated units was obtained when *P. guezennei* was grown on 3 g/L sodium octanoate and 3 g/L 10-undecenoic acid, and with 75% olefinic groups when 3 g/L 10-undecenoic acid was used as the sole carbon source. The high degree of side-chain unsaturation resulted in an amorphous polymer with a consistency of a viscous, sticky, unhandle gum at room temperature. Moreover, these PHOU samples became insoluble in chloroform after a few weeks, probably because of cross-linking reactions caused by the high concentration of unsaturated groups.

TMSi derivatives of 3-hydroxyalkanoic acids methyl esters provide very distinct mocelular ion-related fragments, and clearly reflects their chain length. However, this derivation is not accurate to determine the position of unsaturation along the side chain.

3.2. Determination of double bond position in monounsaturated 3-hydroxyalkenoic acids

GC–MS analysis of thiomethyl acetylated methyl esters was carried out to determine the position of double bond in

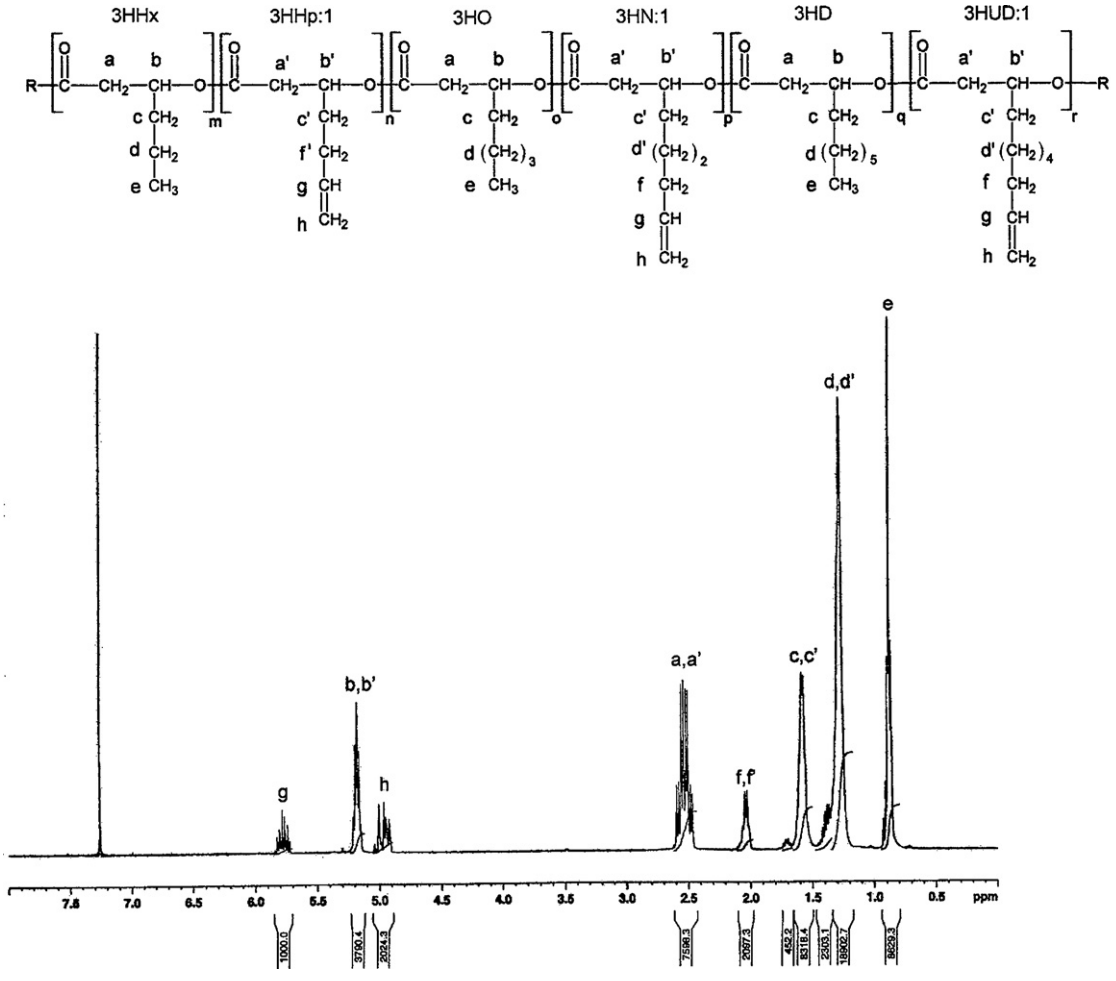

Fig. 6. ^{1}H NMR spectrum in $CDCL_3$ of PHOU containing 26% unsaturated units, produced by *P. guezennei* from 3 g/L sodium octanoate and 1 g/L 10-undecenoic acid. The percentage of olefin groups was calculated from the ratio of peak at 5.7 ppm (g) to peak at 5.2 ppm (b, b′).

monomers constituting mcl-PHAs as well as in monounsaturated 3-hydroxyalkenoic acids standards. The mass spectra of thiomethyl acetylated methyl esters of 3-hydroxyalkenoic acids showed recognizable molecular ions at m/z [$M^{\bullet+}$] and key fragments that allow us to clearly identify the position of the double bond. Key fragments [A^+] and [B^+] derived from the cleavage of the carbon–carbon bond between the two methyl sulfide groups CH_3S as shown in Fig. 2. Fragment [B^+] is often detected in low intensity because it further decomposes and gives [B^+—HOAc] via loss of the O-acetyl group. Additional ions in a lower mass range were detected for [B^+—HOAc] after successive loss of the $HSCH_3$ group and/or acetyl group, thus confirming the olefin group position (Fig. 3).

Reliability of the method was checked using 3-hydroxyalkenoic acids standards with olefinic groups in different positions. The GC–MS patterns of thiomethyl acetylated methyl esters derivatives obtained during this study are summarized in Table 2.

The mass spectra of thiomethyl acetylated methyl esters derivatives of mcl-PHAs produced by *P. guezennei* cultivated on glucose, and on sodium octanoate plus 10-undecenoic acid allowed a complete characterization of the monomeric composition and location of unsaturation. So, the 3HDD:1 unit (as identified by GC–MS analysis of TMSi methyl esters) within mcl-PHAs produced by *P. guezeneni* from glucose was identified as 3HDD:1Δ5, as deduced from its fragmentation pattern showing parent ion [$M^{\bullet+}$] at m/z 364, fragment [A^+] at m/z 145 and [B^+—HOAc] at m/z 159 (Fig. 4).

Concerning PHOU, the same method allowed the determination of the double bond in terminal position as shown by the mass spectra of 3HHp:1Δ6, 3HN:1Δ8 and 3HUD:1Δ10 with a high intensity of fragment [A^+] at m/z 61 ($CH_2{=}^+SCH_3$) together with detection of [B^+—HOAc] at m/z 173, 201 and 229 for 3HHp:1Δ6, 3HN:1Δ8 and 3HUD:1Δ10, respectively, characterizing the SH—CH_3 group in terminal position (Fig. 5, Table 2).

The terminal position of double bond within PHOU was confirmed by NMR analysis, that exhibits signals at 4.9 and 5.7 ppm on the ^{1}H NMR spectra, corresponding to the ethylenic protons of terminal vinylic group (CH=CH_2), whereas signal at 0.9 ppm was attributed to methyne proton (CH_3) in terminal position of saturated side chain (Fig. 6). Moreover no other CH signal was detected on the proton spectra, thus confirming the absence of unsaturation in other location. The percentage of unsaturated units in PHOU was determinated from the ratio of the integration peak at 5.7 ppm (g) to peak at 5.2 ppm (b, b′). It was found 8.8%, 20%, and 26% for concentrations of 10-undecenoic acid of 0.2, 0.5 and 1 g/L, respectively. Those results are consistent with data obtained from GC–MS analysis.

4. Conclusion

We described a novel GC–MS method for the characterization of mcl PHAs with special feature for the determination of olefin group position. The method developed in the present study is suitable for the routine analysis of PHAs since it requires few polymer and short-time analysis while appropriate treatment of the polymer offer characteristic fragments. The method implied a three derivations steps process including a methanolysis, then acetylation onto 3-hydroxy group and thiomethylation on each side of the double bond. The GC–MS analysis of resulting derivatives offered characteristic fragments allowing unambiguously the localization of double bond in monounsaturated 3-hydroxyalkenoic acids.

This technique was validated both on 3-hydroxyalkenoic acids standards as well as on mcl-PHAs produced by *P. guezennei* from different carbons sources. In particular, we described the synthesis of PHOU as a mcl-PHAs containing vinyl-terminated side chain, whose monomeric composition and degree of unsaturated units can be adjusted by changing the concentration of 10-undecenoic acid supplied. mcl-PHAs containing vinyl-terminated side chain with controlled unsaturation degree share interesting properties and can be easily modified due to the terminal position of double bond more accessible for further chemical modifications. Chemical modifications can greatly impart bacterial polyesters properties, and will expand their use in the medical and environmental areas.

Acknowledgments

The authors would like to thank IFREMER and CNRS for financial support.

References

[1] A.J. Anderson, E.A. Dawes, Microbiol. Rev. 54 (1990) 450.
[2] Y. Doi, Microbial Polyesters, VHC Publishers, New-York, 1990.
[3] P.J. Hocking, R.H. Marchessault, in: G.J.L. Griffin (Ed.), Chemistry and Technology of Biodegradable Polymers, Biopolyesters, Chapman and Hall, London, 1994, p. 48.
[4] A. Steinbüchel, H.E. Valentin, FEMS Microbiol. Lett. 128 (1995) 219.
[5] G. Braunegg, G. Lefebvre, K.F. Genser, J. Biotechnol. 65 (1998) 127.
[6] C.R. Hankermeyer, R.S. Tjeerdema, Rev. Environ. Contam. Toxicol. 159 (1999) 1.
[7] D.K.Y. Solaiman, R.D. Ashby, T.A. Foglia, Appl. Microbiol. Biotechnol. 56 (2001) 664.
[8] T. Tsuge, J. Biosci. Bioeng. 94 (2002) 579.
[9] R.D. Ashby, D.K.Y. Solaiman, T.A. Foglia, J. Polym. Environ. 12 (2004) 105.
[10] R.D. Ashby, D.K.Y. Solaiman, T.A. Foglia, Biomacromolecules 6 (2005) 2106.
[11] M. Koller, R. Bona, G. Braunegg, C. Hermann, P. Horvat, M. Kroutil, J. Martinz, J. Neto, L. Pereira, P. Varila, Biomacromolecules 6 (2005) 561.
[12] M. Zinn, B. Witholt, T. Egli, Adv. Drug Deliv. Rev. 53 (2000) 5.
[13] C.W. Pouton, S. Akhtar, Adv. Drug Deliv. Rev. 18 (1996) 133.
[14] S.F. Williams, D.P. Martin, D.M. Horowitz, O.P. Peoples, Int. J. Macromol. 25 (1999) 111.
[15] G.Q. Chen, Q. Wu, Biomaterials 26 (2005) 6565.
[16] M.M. Bear, M.-A. Leboucher-Durand, V. Langlois, R.W. Lenz, S. Goodwin, P. Guerin, React. Funct. Polym. 34 (1997) 65.
[17] N. Kurth, E. Renard, F. Brachet, D. Robic, P. Guérin, R. Bourbouze, Polymer 43 (2002) 1095.
[18] D.J. Stigers, G.N. Tew, Biomacromolecules 4 (2003) 193.
[19] A.H. Arkin, B. Hazer, M. Borcakli, Macromolecules 33 (2000) 3219.
[20] M.S. Eroglu, B. Hazer, T. Ozturk, T. Caykara, J. Appl. Polym. Sci. 97 (2005) 2132.
[21] M.Y. Lee, W.H. Park, W. Lenz, Polymer 41 (2000) 1703.
[22] E. Renard, A. Poux, L. Timbart, V. Langlois, P. Guérin, Biomacromolecules 6 (2005) 891.
[23] J. Babinot, E. Renard, V. Langlois, Macromol. Rapid Commun. 31 (2010) 619.
[24] G.N.M. Huijberts, G. Eggink, P. De Waard, G.W. Huisman, B. Witholt, Appl. Environ. Microbiol. 58 (1992) 536.
[25] P. De Waard, H. van der Wal, G.N.M. Huijberts, G. Eggink, J. Biol. Chem. 268 (1993) 315.
[26] R.J. Sanchez, J. Schripsema, L.F. da Silva, M.K. Taciro, J.G.C. Pradella, J.G.C. Gomez, Eur. Polym. J. 39 (2003) 1385.
[27] P. Furrer, R. Hany, D. Rentsch, A. Grubelnik, K. Ruth, S. Panke, M. Zinn, J. Chromatogr. A 1143 (2007) 199.
[28] G. Braunegg, B. Sonnleitner, R.M. Lafferty, Eur. J. Appl. Microbiol. 6 (1978) 29.
[29] L.L. Wallen, W.K. Rohwedder, Environ. Sci. Technol. 8 (1974) 576.
[30] R.G. Langeveen, G.W. Huisman, H. Preusting, P. Ketelaar, G. Eggink, B. Witholt, Appl. Environ. Microbiol. 54 (1988) 2924.
[31] G.N.M. Huijberts, T.C. De Rijk, P. De Waard, G. Eggink, J. Bacteriol. 176 (1994) 1661.
[32] R.H. Findlay, D.C. White, J. Exp. Mar. Biol. Ecol. 72 (1983) 25.
[33] V. Riis, W. Mai, J. Chromatogr. 445 (1988) 285.
[34] E.Y. Lee, C.Y. Choi, J. Ferment. Bioeng. 80 (1995) 408.
[35] D.W. Johnson, M.-U. Trinh, Rapid Commun. Mass Spectrom. 17 (2003) 171.
[36] P. Scribe, J. Guezennec, J. Dagaut, C. Pepe, A. Saliot, Anal. Chem. 60 (1988) 928.
[37] G.N. Jham, A.B. Attygale, J. Meinwald, J. Chromatogr. A 1077 (2005) 57.