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Short communication

Microwave-induced rapid synthesis of 4-carbethoxyhexafluorobutyryl derivatives of fatty alcohols —a novel derivative for gas chromatography–chemical ionization mass spectrometric study

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Abstract

Structural analyses of fatty alcohols are usually performed as acetate, trifluoroacetate or trimethylsilyl derivatives which produce characteristic molecular ions at $m/z < 400$. We describe a new derivatization technique of fatty alcohol using 4-carbethoxyhexafluorobutyryl chloride. The derivatization reaction requires either 30 min of incubation of the reaction mixture at 60°C, or 4 min of microwave irradiation using 240 W power. The yields of the derivatives were quantitative under both heating condition and microwave irradiation. The 4-carbethoxy hexafluorobutyryl derivatives of fatty alcohols produce characteristic protonated molecular ion peaks in the range of m/z 493 (cetyl alcohol) to m/z 549 (arachidyl alcohol) in the chemical ionization mode using methane as reagent gas. The molecular ion peaks were 54 u more than the conventional heptafluorobutyryl derivatives of fatty alcohols which can also be prepared by microwave irradiation in 3 min (240 W). The new derivatives are less volatile than the conventional heptafluorobutyryl derivatives.

1. Introduction

Fatty alcohols and aldehydes are important natural products found in the lipids of plants, animals and bacteria [1–4]. Surface lipids usually contain wax esters (fatty alcohols esterified to fatty acids), non-esterified fatty acids and sterols. Free and esterified fatty alcohols are also abundant in germinating seeds and in marine organisms, for example, oil of the deep sea fish orange roughy (*Hoplostethus atlanticus*) contains 95% wax esters. The presence of mycobacteria in drinking water represents a significant public health problem. Hydrolysis of those wax esters isolated from bacteria produces characteristic fatty alcohols which are derivatized and analyzed by gas chromatography–mass spectrometry (GC–MS) for identification [5,6]. The Sjogren–Larsson syndrome, an autosomal recessive disorder, is due to a defect in fatty alcohol cycle. Cultured skin fibroblast of these patients accumulate fatty alcohols and analysis of fatty alcohols after derivatization is used as a diagnostic aid for this disease [7,8]. Long-chain fatty acyl coenzyme thioester A have been implicated as substrates for several metabolic reactions. The assay of these compounds can be performed by taking advantage of borohydride reduction of

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these thioesters to fatty alcohols followed by derivatization of fatty alcohols for further analysis [9]. Therefore, analysis of fatty alcohols is widely used in lipid biochemistry.

Fatty alcohols are usually analyzed after conversion to acetate, trifluoroacetate or trimethylsilyl derivatives. These derivatives produce molecular ions at $m/z < 400$. Czarny and Hornbeck [10] recently described derivatization of amphetamine and methamphetamine using 4-carbethoxyhexafluorobutyryl chloride. We studied the derivatization of fatty alcohols using this reagent, hypothesizing the production of molecular ions in a much higher range (492–548).

Recently, microwave irradiation was demonstrated to produce dramatic acceleration of reaction rates of a variety of reactions [11–14]. We previously reported microwave-induced rapid transesterification of lipids and accelerated synthesis of fatty acyl pyrrolidides [15]. We also reported microwave-induced rapid preparation of conventional acetate and trifluoroacetate derivatives of fatty alcohols [16]. Now we would like to report microwave-induced rapid preparation of a novel 4-carbethoxyhexafluorobutyryl derivative of fatty alcohols.

2. Experimental

Cetyl, oleyl, linoleyl, stearyl, arachidonyl and arachidyl alcohols, 11-eicosenol and the internal standard, heptadecanol were purchased from Sigma (St. Louis, MO, USA). The derivatizing agent 4-carbethoxyhexafluorobutyryl chloride was obtained from PCR (Gainsville, FL, USA), while heptafluorobutyric anhydride was procured from Pierce (Rockford, IL, USA). The derivatization reaction was carried out in Reacti-Vials with 1 ml capacity capped with Mini Inert valves, also available from Pierce.

The high-performance thin-layer chromatography (HPTLC) plates coated with silica gel were obtained from EM Separation (Gibbstown, NJ, USA). The developing solvent was ethyl acetate–methanol (90:10, v/v). After developing, bands were visualized by spraying with 4% copper sulfate in 30% phosphoric acid followed by heating. The microwave oven used in this study has a total capacity of 800 W with ten different power settings (Samsung, Model MW 5510 T). The GC–MS analysis was performed by using a Model 5890 gas chromatograph coupled with a 5970 series mass-selective detector for electron impact (EI) MS study and a 5890 series II gas chromatograph coupled to a 5972 series mass-selective detector for chemical ionization (CI) spectra (Hewlett-Packard, Palo Alto, CA, USA). An Ultra-2 capillary column, also obtained from Hewlett-Packard, was used for both instruments. The initial oven temperature of the gas chromatograph was 190°C for analysis of carbethoxyhexafluorobutyryl derivatives of fatty alcohols. After maintaining that temperature for 2 min, the temperature of the gas chromatograph was increased at a rate of 5°C/min to reach a final oven temperature of 290°C. The final temperature was maintained for another 2 min. The solvent delay was 5 min after which the mass spectrometer was turned on. The carrier gas was helium with a column flow-rate of 0.29 ml/min and a linear velocity of 21 cm/s. For the analysis of heptafluorobutyryl derivatives, the initial oven temperature was 160°C. After maintaining that temperature for 2 min, the oven temperature was increased at a rate of 5°C/min to reach a final oven temperature of 290°C. The final oven temperature was maintained for an additional 2 min and the solvent delay was again 5 min. Both mass spectrometers were operated in the scan mode with scanning range of m/z 50–700.

2.1. Preparation of 4-carbethoxyhexafluorobutyryl derivatives

We added 50 μl of 4-carbethoxyhexafluorobutyryl chloride to 0.1–0.2 mg of fatty alcohol and heated the reaction mixture at 60°C for 30 min. Finally the reaction mixture was evaporated to dryness. We took advantage of low volatility of the derivatized fatty alcohol and omitted the addition of anhydrous ethanol to the reaction mixture as described by Czarny and Hornbeck [10]. Instead we evaporated the excess reagent at 37°C and reconstituted the residue in ethyl acetate and injected into GC–MS. Using micro-

wave irradiation (power level 3, 240 W), the reaction was completed only in 4 min.

2.2. Preparation of heptafluorobutyryl derivatives

We also prepared conventional heptafluorobutyryl derivatives of fatty alcohols in 3 min using low power microwave irradiation (power level 3, 240 W). The conventional heating technique requires 20 min incubation of reaction mixture at 60°C. The reaction was quantitative using both microwave irradiation and conventional heating method as evidenced by the complete disappearance of the starting material in HPTLC plates.

3. Results and discussion

3.1. Microwave-induced rapid derivatization

The microwave provides a rapid and convenient method for preparation of 4-carbethoxyhexafluorobutyryl derivatives of fatty alcohols for structural analysis. The R_F values of fatty alcohols obtained by the microwave technique were identical to those of the derivatives obtained by conventional heating. The GC retention times as well as MS fragmentation patterns of the derivatives prepared by microwave irradiation were similar to those of the derivatives obtained by conventional heating, indicating that the derivatives obtained by the microwave technique have the same chemical identity as the derivatives prepared by conventional heating (Table 1).

We reported previously the use of microwave irradiation for rapid preparation of acetyl, trifluoroacetyl and *tert.*-butyltrimethylsilyl derivatives of fatty alcohols [16]. We prepared heptafluorobutyryl derivatives of fatty alcohols in 3 min under microwave irradiation (power 3, 240 W) while the conventional technique requires 20 min of heating at 60°C. Again, the GC retention times and MS fragmentation patterns of derivatives formed under microwave irradiation were identical to those obtained by the conventional heating technique.

Table 1
CI-MS characteristics of 4-carbethoxyhexafluorobutyryl derivatives of fatty alcohols prepared by microwave irradiation and conventional heating

Compound	MS fragmentation pattern							
	Microwave[a]				Heating[a]			
	M + 1	Base	Other peaks		M + 1	Base	Other peaks	
Cetyl alcohol	493 (5)	225 (100)	169 (8)	155 (13)	493 (4)	225 (100)	169 (8)	155 (13)
Stearyl alcohol	521 (2)	253 (100)	169 (12)	155 (14)	521 (2)	253 (100)	169 (12)	155 (13)
Oleyl alcohol	519 (14)	111 (100)	251 (31)	167 (47)	519 (16)	111 (100)	251 (31)	167 (46)
Linoleyl alcohol[b]	517 (12)	97 (100)	249 (35)	165 (45)	517 (11)	97 (100)	249 (36)	165 (45)
Arachidyl alcohol	549 (2)	281 (100)	183 (11)	169 (13)	549 (3)	281 (100)	183 (9)	169 (11)
Eicosenol	547 (11)	97 (100)	181 (29)	167 (44)	547 (12)	97 (100)	181 (31)	167 (45)
Arachidonyl alcohol	541 (100)	541 (100)	273 (20)	137 (33)	541 (100)	541 (100)	273 (20)	137 (32)
Heptadecanol (I.S.)	507 (1)	239 (100)	183 (8)	169 (11)	507 (1)	239 (100)	183 (7)	169 (10)

I.S. = Internal standard.
[a] m/z, relative abundance in parentheses.
[b] For linoleyl alcohol the M − 1 peak at m/z 515 (relative abundance 27.1%) was stronger than the M + 1 peak.
× I.S. stands for internal standard

3.2. GC retention times and MS characteristics of fatty alcohol derivatives

The advantage of derivatizing fatty alcohols with 4-carbethoxyhexafluorobutyryl chloride is the significantly lower volatility of these derivatives compared to the conventional heptafluorobutyryl derivatives, while retaining excellent chromatographic properties (no significant tailing of peaks). For example, if we set the oven temperature of our gas chromatograph at 160°C, the temperature program we used for the analysis of heptafluorobutyryl derivatives, the retention time of the heptafluorobutyryl derivative of arachidyl alcohol was 16.1 min while the retention time of 4-carbethoxyhexafluorobutyryl derivative of arachidyl alcohol was 24.5 min. The lower volatility of these new derivatives will be helpful in analysis of short- and medium-chain alcohols as well as for fatty alcohols where higher initial oven temperatures should be used. We used heptadecanol as the internal standard.

Another advantage of 4-carbethoxyhexafluorobutyryl derivatives of fatty alcohols is the significant increase in the molecular ion peak compared to the conventional derivatives. For example, the molecular ion peaks of acetate and trifluoroacetyl derivatives of arachidonyl alcohol were observed at m/z 332 and 386, respectively [16]. The heptafluorobutyryl derivative of arachidonyl alcohol (a derivative less commonly used) showed a molecular ion peak at m/z 486, while the 4-carbethoxyhexafluorobutyryl derivative showed a molecular ion peak + 1 at m/z 541 (Fig. 1).

The acetyl, trifluoroacetyl or heptafluorobutyryl derivatives of saturated fatty alcohols (cetyl, stearyl and arachidyl) did not show any molecular ion peak in the EI mode [16]. However, 4-carbethoxyhexafluorobutyryl derivatives of saturated fatty alcohols showed weak molecular ion peaks (Fig. 2). The more intense peaks in the EI mode for both heptafluorobutyryl and 4-carbethoxyhexafluorobutyryl derivatives of fatty alcohols were observed between m/z 50–100, while the peaks in the higher mass range showed much lower relative abundances. However, in the CI mode using methane as a reagent gas more intense peaks can be observed in the higher mass range.

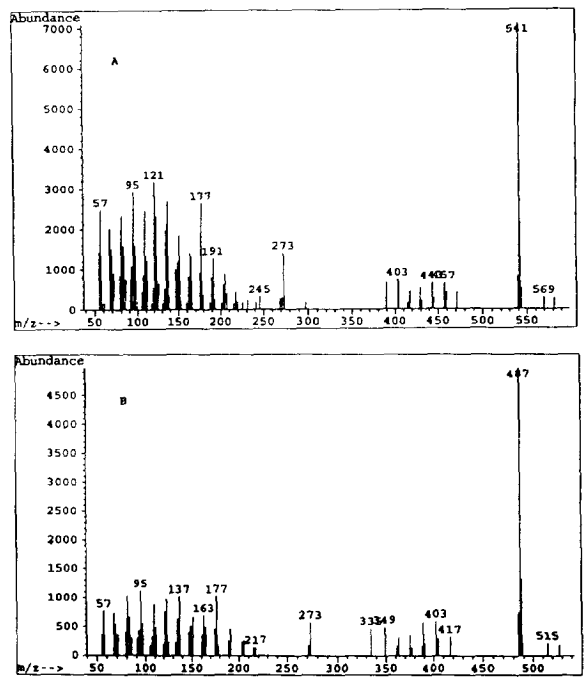

Fig. 1. Chemical ionization mass spectra (methane as a reagent gas) of (A) 4-carbethoxyhexafluorobutyryl derivative and (B) heptafluorobutyryl derivative of arachidonyl alcohol.

Both heptafluorobutyryl and 4-carbethoxyhexafluorobutyryl derivatives of fatty alcohols (saturated and unsaturated) showed distinct protonated and/or M − 1 peak peaks in the CI mode. Another advantage of CI-MS analysis is the distinctively different fragmentation patterns of saturated and unsaturated alcohols. The saturated fatty alcohols after derivatization either with heptafluorobutyryl anhydride or 4-carbethoxyhexafluorobutyryl chloride, showed the same base peaks due to the loss of derivatized part of the molecule including oxygen. We also observed several peaks due to fragmentation of the hydrocarbon part of the molecule after the loss of derivatized part which were similar in both derivatives. As expected, the molecular ion peaks are 54 u higher in 4-carbethoxyhexafluorobutyryl derivatives compared to the heptafluorobutyryl derivatives. The unsaturated al-

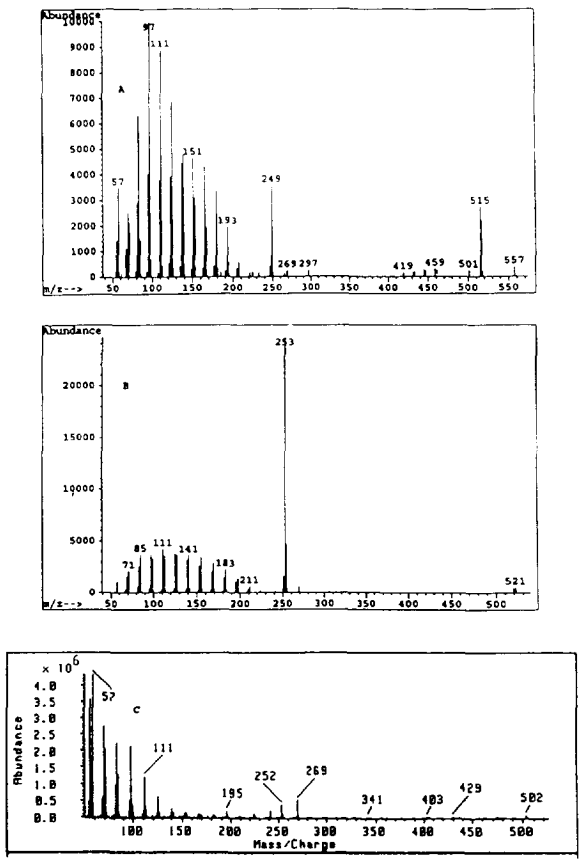

Fig. 2. Chemical ionization mass spectra (methane as a reagent gas) of 4-carbethoxyhexafluorobutyryl derivatives of (A) linoleyl alcohol, (B) stearyl alcohol and (C) stearyl alcohol in the electron impact mode.

cohols, after derivatization, showed much stronger protonated M − 1 peaks while the base peaks were shifted to the lower mass region. However, the peaks in the smaller mass regions of derivatized unsaturated alcohols were more intense than the derivatized saturated alcohols, thus distinguishing MS characteristics of unsaturated alcohols from saturated alcohols. This distinct difference between the MS fragmentation pattern of derivatized saturated and unsaturated alcohol is absent in the EI mode where both saturated and unsaturated compounds showed intense peaks in the m/z 50–100 region. We also observed a distinct peak in the mass spectrum of derivatized unsaturated alcohols due to the loss of the derivatized part of the molecule including oxygen. The straight-chain and branched-chain fatty alcohols after derivatization with 4-carbethoxyhexafluorobutyryl chloride showed different intensities of peaks in the CI mode. The heptafluorobutyryl derivatives of saturated and unsaturated alcohols also showed these distinguishing features in the CI mass spectra using methane as a reagent gas.

The CI- and EI-MS analysis for derivatized fatty alcohol provide complementary information. A distinct feature in the CI mode is the observation of strong molecular ions peaks for unsaturated alcohols for both heptafluorobutyryl and 4-carbethoxyhexafluorobutyryl derivatives. In addition, both derivatives of saturated fatty alcohols showed molecular ion peaks in the CI mode while molecular ion peaks were not observed for heptafluorobutyryl derivatives in the EI mode, although 4-carbethoxyhexafluorobutyryl derivatives showed weak molecular ion peaks in the EI mode.

We conclude that microwave irradiation can be utilized for rapid synthesis of new 4-carbethoxyhexafluorobutyryl or conventional heptafluorobutyryl derivatives of fatty alcohols for structural analysis.

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