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Use of microwave irradiation for rapid synthesis of perfluorooctanoyl derivatives of fatty alcohols, a new derivative for gas chromatography–mass spectrometric and fast atom bombardment mass spectrometric study

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Abstract

Structural analyses of fatty alcohols as acetate, trifluoroacetate and trimethylsilyl derivatives are frequently used in lipid biochemistry. Those derivatives produce molecular ions (m/z) < 400. Perfluorooctanoyl derivatives of fatty alcohols which can produce ions in the range of 600–700 (m/z) have never been studied before. We prepared perfluorooctanoyl derivatives of fatty alcohols by heating them with perfluorooctanoyl chloride for 15 min at 60°C. Using low-power microwave irradiation (240 W), we can reduce the reaction time to only 2 min. The yields of the derivatives were quantitative by both microwave technique and conventional heating as evidenced by absence of starting material (fatty alcohols) in the HPTLC analysis. The mass spectral fragmentation patterns of the derivatives obtained by microwave irradiation are identical to the derivatives prepared by conventional heating. We also prepared trimethylsilyl derivatives of fatty alcohols, a widely used derivative, in 1.5 min using microwave irradiation (power 3, 240 W) where the conventional technique requires 20 min. We conclude that microwave irradiation can be employed for rapid preparation of perfluorooctanoyl and trimethylsilyl derivatives of fatty alcohols for gas chromatography–mass spectrometric analysis.

1. Introduction

Fatty alcohols and aldehydes have been isolated from lipids of bacteria, animals and plants [1–4]. Surface lipids usually contain a complex mixture of fatty acids esterified to fatty alcohols (wax esters), hydrocarbons, non-esterified fatty acids and sterols. Free and esterified fatty alcohols are also found in large quantities in germinating seeds for the purpose of energy storage. Wax esters are also abundant in marine organisms, for example the oil of the sperm whale contains 70% wax ester while the oil of deep sea fish orange roughy contains 95% wax esters. For analysis, wax esters are hydrolyzed to liberate fatty acids and fatty alcohols. After separation, fatty alcohols are derivatized and analyzed by gas chromatography (GC) or GC–mass spectrometry (MS) [5,6].

The presence of mycobacteria in drinking

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water represents a significant public health problem. Hydrolysis of wax esters from those mycobacteria produces characteristic fatty alcohols which are derivatized and analyzed by GC–MS for identification of those bacteria in drinking water [7,8]. The Sjogren–Larsson syndrome, an autosomal recessive disorder is due to the defect in fatty alcohol cycle. Affected patients usually survive childhood, but life expectancy is decreased. Cultured skin fibroblast of these patients accumulate fatty alcohols and analysis of fatty alcohol after derivatization is used as a diagnostic aid for this disease [9,10].

Long-chain fatty acyl coenzyme A thioesters have been implicated as substrates for several metabolic reactions [11] and their concentrations can be increased in many pathological conditions like ischemia. The assay of those compounds can be performed by taking advantage of borohydride reduction of those thioesters to fatty alcohols [12].

Usually, fatty alcohols are analyzed after converting them to acetate, trifluoroacetyl or trimethylsilyl derivatives. Those derivatives usually produce characteristic ions in the mass range < 400. Recently, derivatization of amphetamine and methamphetamine with perfluorooctanoyl chloride have been described [13]. We studied the possibility of derivatizing fatty alcohols with that reagent in order to produce ions in the much higher mass region 600–700 for unambiguous structural analysis. To our knowledge, this particular derivative of fatty alcohols has not been reported before.

Recently, microwave irradiation was demonstrated to produce dramatic acceleration of reaction rates. Dayal et al. [14] described a method for rapid hydrolysis of bile acid methyl esters using a commercially available microwave oven. Microwave-induced rapid acceleration of reaction rates for Diels–Alder, Ene, Claisen reaction, hydrolysis of proteins and peptides had been described [15–17]. The use of microwave irradiation for rapid epoxidation of fatty acid esters, cyclization of dioxostearates and oxounsaturated fatty acids esters into furanoid derivatives, conversion of epoxystearate to oxostearate and substitution of a tosyl group by an azide group have also been described in the literature [18]. We previously reported microwave-induced rapid transesterification of lipids and accelerated synthesis of fatty acyl pyrrolidides for GC–MS study [19]. We also reported the use of microwave oven for rapid preparation of conventional acetate and trifluoroacetyl derivatives of fatty alcohols [20]. Now we would like to report synthesis of perfluorooctanoyl derivative of fatty alcohols, a new derivative, and conventional trimethylsilyl derivatives using microwave irradiation.

2. Experimental

Cetyl, stearyl, oleyl, linoleyl, arachidonyl, arachidyl alcohol and eicosenol were purchased from Sigma (St. Louis, MO, USA). The derivatizing agent bis(trimethylsilyl)trifluoroacetamide was also purchased from Sigma while perfluorooctanoyl chloride was procured from PCR (Gainsville, FL, USA). The derivatizing reactions were carried out in reaction vials with a total capacity of 1 ml (Pierce, Rockford, IL, USA). The vials were capped with mini inert valves also available from Pierce.

The HPTLC plates coated with silica were obtained from EM Separations (Gibbstown, NJ, USA). The developing solvent was hexane–ethyl acetate (65:35). 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 (Samsung, Model MW 5510 T). The GC–MS analysis was carried out using a Model 5890 gas chromatograph coupled with 5970 series mass-selective detector (Hewlett-Packard, Palo Alto, CA, USA). The capillary column used was an Ultra-2, also available from Hewlett-Packard. The initial oven temperature of the gas chromatograph was 180°C. After maintaining the temperature for 2 min, the temperature was increased at a rate of 4°C/min to reach an oven temperature of 240°C. Then the

oven temperature was increased at a rate of 10°C/min to reach a final oven temperature of 280°C. The mass spectrometer was operated in a scan mode with a scan range of 40–800 m/z, or a scan range of 200–800 m/z.

The fast atom bombardment (FAB) MS study was performed using a Model VG 70-SEQ mass spectrometer manufactured by Varian Instruments. The matrix used for this study was 3-nitrobenzoyl alcohol. The samples were bombarded with xenon atom beams with 8 keV energy.

2.1. Preparation of perfluorooctanoyl derivatives

We added 50 μl of derivatizing reagent to 0.01–0.05 mg of the fatty alcohol. The reaction was also complete in 2 min under microwave irradiation (power 3, 240 W). Gjerde et al. [13] recommended 30 min of heating at 60°C for conversion of amphetamine and methamphetamine to their corresponding perfluorooctanoyl derivative. Our study indicated that quantitative conversion of fatty alcohol to perfluorooctanoyl derivative can be achieved by heating the reaction mixture at 60°C for 15 min. After the reaction, excess derivatizing reagents were evaporated to dryness and the residue was reconstituted in ethyl acetate and injected into the GC–MS system.

2.2. Preparation of trimethylsilyl derivatives

We added 300 μl of bis(trimethylsilyl)trifluoroacetamide to 0.1–0.2 mg of fatty alcohol. Under microwave irradiation, the reaction was completed in 1.5 min under low power (power 3, 240 W) as evidenced by complete disappearance of starting material in the HPTLC analysis. For conventional conditions, the reaction mixture was allowed to stand at the room temperature for 20 min. Since fatty alcohols are stable only in excess derivatizing agent no work-up was performed. The reaction mixture was directly injected into the GC–MS system.

3. Results and discussion

3.1. Microwave technique for rapid derivatization

The microwave provides a rapid and convenient method for preparation of perfluorooctanoyl derivatives of fatty alcohols for structural analysis by GC–MS. The R_F values by HPTLC of derivatives prepared by microwave irradiation and conventional technique are always the same. Analysis of reaction products after microwave irradiation by HPTLC showed quantitative conversion of fatty alcohols to the perfluorooctanoyl derivatives. We also did not observe any undesirable decomposition products. The quantitative conversion of fatty alcohols to the corresponding derivatives either by microwave irradiation or conventional heating was also evidenced by the absence of underivatized alcohol peaks in the gas chromatogram. Moreover, the GC retention times and MS fragmentation patterns of fatty alcohol derivatives prepared by microwave irradiation were identical to those fatty alcohol derivatives prepared by conventional heating. Therefore, microwave irradiation produced derivatives which have the same chemical identity as derivatives produced by conventional heating (Table 1, Fig. 1).

We also use microwave irradiation for rapid derivatization of fatty alcohols with bis(trimethylsilyl)trifluoroacetamide because trimethylsilyl derivatives of fatty alcohols are widely used by investigators in lipid biochemistry. Weppelman et al. [21] recommended 20 min incubation of reaction mixture at room temperature for complete conversion to trimethylsilyl derivatives while Myher and Kuksis [22] recommended an incubation period of 30–60 min for complete conversion. Our results indicate that reaction was complete in 20 min. On the other hand the conversion of fatty alcohols to the corresponding trimethylsilyl derivatives was complete in 90 s under low-power microwave irradiation (power level 3, 240 W). The R_F value of trimethylsilyl derivatives of fatty alcohols prepared by microwave irradiation was the same as

Table 1
Mass spectral characterization of perfluorooctanoyl derivatives of fatty alcohols prepared by microwave irradiation and conventional heating

Compound	Mass spectral fragmentation pattern					
	Microwave			Conventional heating		
	M^{+} [a]	Base[a]	Other peaks[a]	M^{+} [a]	Base[a]	Other peaks[a]
Cetyl	–	43 (100)	620 (1), 224 (10)	–	43 (100)	620 (1), 224 (8)
Stearyl	–	43 (100)	648 (1), 252 (13)	–	43 (100)	648 (1), 252 (9)
Oleyl	664 (9)	55 (100)	578 (1), 369 (2)	664 (7)	55 (100)	578 (1), 369 (2)
Linoleyl	662 (15)	67 (100)	578 (3), 564 (4)	662 (13)	67 (100)	578 (3), 564 (3)
Arachidyl	–	57 (100)	676 (1), 479 (1)	–	57 (100)	676 (1), 479 (1)
11-Eicosenol	692 (3)	55 (100)	578 (1), 369 (1)	692 (3)	55 (100)	578 (1), 369 (1)
Arachidonyl	686 (2)	79 (100)	588 (9), 548 (9)	686 (2)	79 (100)	588 (8), 548 (8)

[a] m/z Values; relative intensities in parentheses.

the derivatives prepared by conventional technique. Similarly, the GC retention times were also similar. The MS fragmentation patterns were also identical again indicating that trimethylsilyl derivatives prepared by microwave irradiation had the same chemical identities as the derivatives prepared by the conventional technique (Table 2).

3.2. Electron impact and FAB mass spectra of fatty alcohol derivatives

The major advantage of derivatizing fatty alcohol with perfluorooctanoyl chloride is the significant increase in the molecular ion peak. For example, the perfluorooctanoyl derivative of arachidonyl alcohol (20:4) showed a molecular

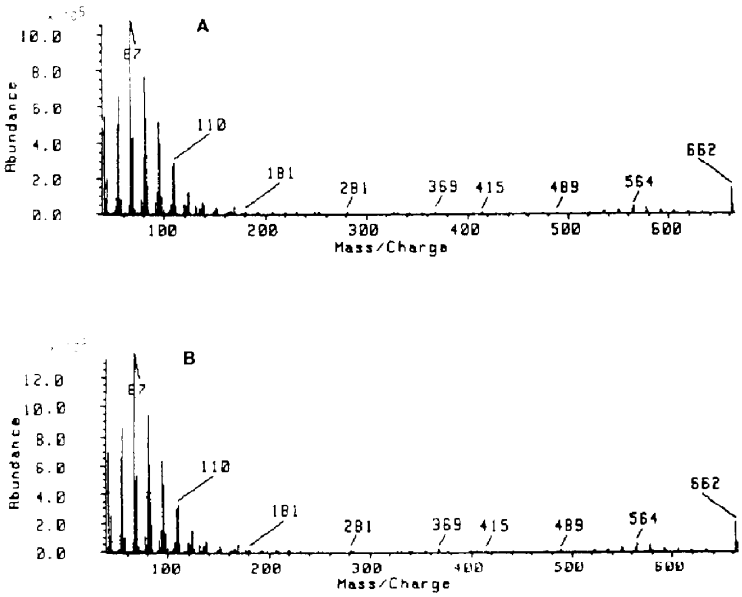

Fig. 1. Mass spectral fragmentation patterns of the perfluorooctanoyl derivative of linoleyl alcohol prepared by (A) conventional heating and (B) microwave irradiation.

Table 2
Mass spectral characterization of trimethylsilyl derivatives of fatty alcohols prepared by microwave irradiation and conventional heating

Compound	Mass spectral fragmentation pattern					
	Microwave			Conventional heating		
	$M^{\cdot}$ [a]	Base[a]	Other peaks[a]	$M^{\cdot}$ [a]	Base[a]	Other peaks[a]
Cetyl	299 (100)	299 (100)	314 (1), 75 (24)	299 (100)	299 (100)	314 (1), 75 (29)
Stearyl	327 (100)	327 (100)	342 (1), 75 (26)	327 (100)	327 (100)	342 (1), 75 (30)
Oleyl	325 (29)	75 (100)	340 (8), 297 (8)	325 (25)	75 (100)	340 (7), 297 (6)
Linoleyl	323 (12)	75 (100)	338 (3), 295 (2)	323 (14)	75 (100)	338 (4), 295 (2)
Arachidyl	355 (100)	355 (100)	339 (1), 75 (23)	355 (100)	355 (100)	339 (1), 75 (30)
11-Eicosenol	353 (31)	75 (100)	368 (11), 325 (10)	353 (29)	75 (100)	368 (9), 325 (8)
Arachidonyl	347 (1)	79 (100)	362 (2), 271 (2)	347 (1)	79 (100)	362 (2), 271 (1)

[a] m/z Values; relative intensities in parentheses.

ion peak at m/z 686 while the conventional trimethylsilyl derivative showed a weak M − 15 peak at m/z 347. In our previous study with microwave-induced rapid preparation of acetyl and trifluoroacetyl derivatives of fatty alcohols, the molecular ion peaks of arachidonyl alcohol were observed only at m/z 332 and m/z 386 respectively [20].

The strongest peaks in the perfluorooctanoyl derivatives of fatty alcohols were observed in the lower mass region (m/z 40–100) while the peaks in the higher mass region (m/z 200–600) showed much lower relative abundances. This feature is also common to other conventional fluoro derivatives of fatty alcohols, for example, the molecular ion peak (m/z 686) and peaks at m/z 588 and m/z 548 in the mass spectrum of the perfluorooctanoyl derivative of arachidonyl alcohol showed relative abundances of 2, 9 and 8%, respectively, relative to the base peak at m/z 79. The relative abundances of molecular ion peak (m/z 386) and peaks at m/z 288 and 248 in the mass spectrum of the trifluoroacetyl derivative of the same alcohol were 3, 10 and 12%, respectively, relative to the base peak at m/z 79 [20]. The trimethylsilyl derivative of arachidonyl alcohol also showed a base peak at m/z 79. However, the M − 15 peak at m/z 347 has a relative abundance of only 1% and the two other peaks at higher mass range at m/z 362 and m/z 271 had relative abundances of only 2% each.

In order to circumvent this problem, we studied FAB mass spectra of perfluorooctanoyl derivatives of fatty alcohols. Interestingly, saturated alcohols showed a weak M − 1 ion peak while any molecular ion or M − 1 peaks were absent in the electron impact mass spectra for those alcohols. The molecular ion peaks are also absent in conventional trifluoroacetyl or acetate derivatives of fatty alcohols [20]. Therefore, FAB-MS analysis has a distinct advantage over conventional electron impact for unambiguous determination of molecular mass of saturated alcohol. We also observed molecular ion peaks and M − 1 peaks in the FAB mass spectra of unsaturated alcohols. With some derivatized alcohol, specially arachidonyl alcohol, the M − 1 peak at m/z 685 was even stronger than molecular ion peak at m/z 686 (Fig. 2). We observed peaks with slightly higher relative abundances in the higher range of mass spectrum in FAB mode compared to the electron impact mode, but again the strongest peaks were observed in the lower mass range ($m/z < 200$) as observed with the electron impact mass spectra (Fig. 2). We did not observe any additional strong diagnostic peak in the higher mass range in the FAB spectra compared to electron impact spectra. Therefore, for unsaturated alcohols, FAB mass

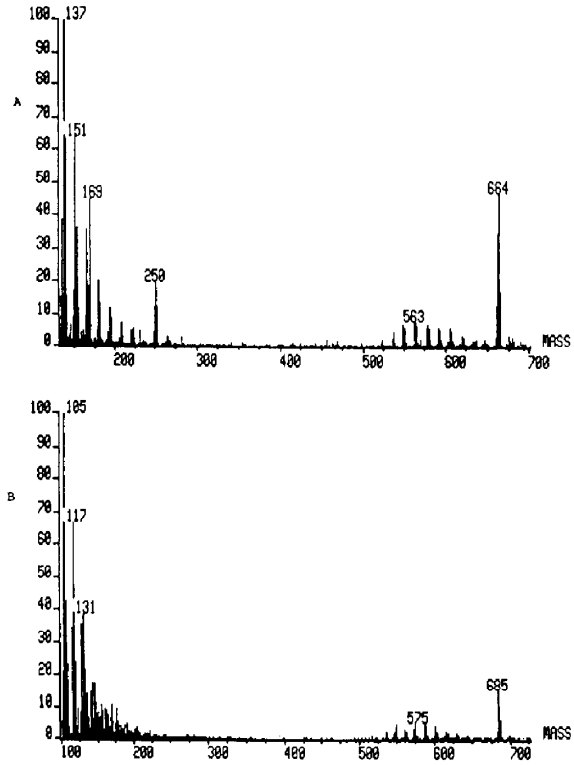

Fig. 2. Fast atom bombardment mass spectrum of perfluorooctanoyl derivatives of (A) oleyl alcohol and (B) arachidonyl alcohol.

spectra did not have any distinct advantage over the conventional electron impact mass spectra for structural analysis.

Since a FAB facility is not available to all laboratories, we studied the possibility of scanning the mass spectrometer of our GC–MS system from m/z 200 to 800 in order to increase the intensities of peaks in the higher mass range. We observed the same fragmentation patterns for all compounds as expected compared to spectra obtained by scanning the mass spectrometer from m/z 40 to 800. However, we observed significant increases in the relative abundances of all peaks in the higher mass ranges for all alcohols. For all unsaturated alcohols, except for arachidonyl alcohol the molecular ion peaks became the base peak. Other characteristic peaks in the 500–650 regions were more prominent when the mass spectrometer was scanned from m/z 200 to 800. For example, the molecular ion peak for arachidonyl derivative has a relative abundance of 40.1% when the mass spectrometer was scanned from m/z 200 to 800 compared to the relative abundance of 2% when scanning from m/z 40 to 800. Similarly, the diagnostic peaks at m/z 588 and m/z 548 had relative abundances of 97.7 and 100% compared to the relative abundances of 8.0 and 7.9%, respectively, when the mass spectrometer was scanned from m/z 40 to 800. Our results showed an excellent signal-to-noise ratio for those diagnostic peaks in the higher mass range when the mass spectrometer was scanned from m/z 200 to 800 (Fig. 3). However the sensitivity of detection was lower when we operated our mass spectrometer in that mode.

3.3. *Perfluorooctanoyl versus trimethylsilyl derivatives*

The major advantage of derivatizing fatty alcohols with perfluorooctanoyl chloride is the significant increase in the molecular ion peak in the mass spectrum as well as lower volatility of the derivative which can aid in eliminating interfering peaks in the gas chromatogram. Extracts from serum or another biological matrix often contains relatively volatile compounds that show up in the lower temperature zone of a gas chromatogram.

The molecular ion peaks of perfluorooctanoyl derivatives of unsaturated fatty alcohols showed higher abundance than saturated fatty alcohols (M − 1 peaks were present only in the FAB mode). On the other hand M − 15 peaks were very strong in the trimethylsilyl derivatives of saturated fatty alcohols, while the peaks were relatively weak with unsaturated alcohols (Tables 1 and 2). Because of these opposite characteristics in the molecular ion peaks for saturated versus unsaturated alcohol, perfluorooctanoyl and trimethylsilyl derivatives can provide complementary structural information for identification of fatty alcohols.

Our results clearly indicate that microwave irradiation is a rapid and convenient way of preparing perfluorooctanoyl and trimethylsilyl

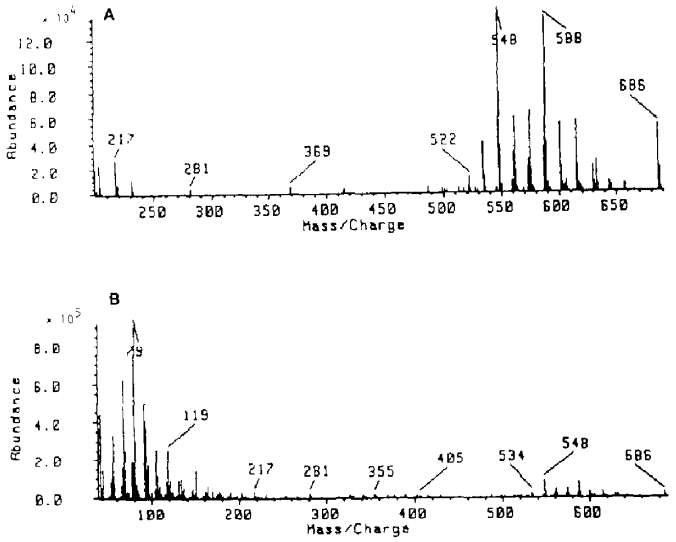

Fig. 3. Mass spectral characteristics of the perfluorooctanoyl derivative of arachidonyl alcohol. (A) Scan from m/z 200 to 800; (B) scan from m/z 40 to 800.

derivatives of fatty alcohols for structural identifications. Moreover, perfluorooctanoyl derivatives of fatty alcohols, a derivative not studied before, offer unique advantages of longer retention times and more characteristic peaks in higher mass region for identification purpose.

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