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Gas–liquid chromatography–mass spectrometry of primary and secondary fatty alcohols and diols as their *tert.*-butyldimethylsilyl derivatives

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Abstract

The use of gas–liquid chromatography (GLC) and mass spectrometry (MS) with derivatizing agents which give stable derivatives and informative fragmentation patterns allows for accurate identification of a variety of compounds. In this comprehensive study, the *tert.*-butyldimethylsilyl (*t*BDMS) derivatives of saturated and unsaturated primary alcohols, iso- and anteiso-primary alcohols, branched alcohols, secondary alcohols and primary diols were produced and analyzed by GLC–MS. Most derivatized alcohols produced prominent mass minus 57 $[M-57]^+$ fragment ions, which, combined with their respective retention data and other key fragment ions, allows for their identification and analysis.

Keywords: Derivatization, GC; Fatty alcohols; Lipids; Diols; Alcohol

1. Introduction

Fatty alcohols occur widely in nature [1] and have been detected as components of lipids from bacteria, insects, animals and plants [1–5] and play a large variety of important roles in nature including structure, cell wall membranes, and as sources of energy. Fatty alcohols can occur as free alcohols, as wax esters and as alkoxy lipids. They may exist in small quantities or, as in some instances, in large amounts, such as observed in the sperm whale and in deep sea orange roughy fish where wax esters may constitute 70% and 90%, respectively, of their oil. Because of their diversity, efforts have been made toward developing convenient and sensitive analytical methods for identifying and quantifying fatty alcohols from different sources. These include acetylation [6,7], trimethylsilylation (TMS) [8–10], trifluoroacetylation [6] and perfluorooctanoylation [11]. Though, the use of *tert.*-butyldimethylsilyl (*t*BDMS) ethers and esters have been shown to be useful in the identification and analysis of amino acids [12,13], fatty acids [14,15], mono-, di- and tricarboxylates [14], hydroxy fatty acids and their methyl esters [15,16], and thiols [17], its use in the analysis of fatty alcohols has been limited to a few compounds [6].

This comprehensive investigation reports an analytical method for the analysis of saturated and unsaturated primary alcohols, iso- and anteiso-primary alcohols, secondary alcohols and primary diols as their stable *tert.*-butyldimethylsilyl derivatives (*t*BDMS). Derivatization is accomplished in a single

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step employing either N-methyl-N-*tert.*-butyldimethylsilyltrifluoroacetamide (MTBSTFA) or MTBSTFA with N-*tert.*-butyldimethyl-silylimidazole (TBSIM) yielding stable, purifiable *t*BDMS-ethers amenable to capillary gas–liquid chromatography (GLC) analysis and detected by flame ionization (FID) and by combined gas–liquid chromatography–mass spectrometry (GLC–MS). Each of the 100 derivatized fatty alcohols tested displayed a single chromatographic GLC peak and, in mixture, most were readily resolved by capillary GLC. The mass spectra for these derivatized alcohols are relatively simple, displaying prominent and unambiguous mass minus 57 $[M-57]^+$ fragment ions, as well as other important fragment ions, that yield information regarding molecular mass, degree of unsaturation, location of alcohol and of any alkyl branches that may be present. Application of this method to a complex mixture of fatty alcohols isolated from New Zealand orange roughy fish illustrates the versatility of this method in the concomitant identification of the various alcohol types.

2. Experimental

2.1. Materials

All primary unbranched fatty alcohols were purchased from Sigma (St. Louis, MO, USA) or generated from the respective fatty acid methyl ester via treatment with lithium aluminum hydride in tetrahydrofuran. Iso- and anteiso-fatty alcohols were similarly synthesized from the methyl ester fatty acid analogs purchased from Matreya (Pleasant Gap, PA, USA). Alkyl diols and secondary alcohols were purchased from Aldrich (Milwaukee, WI, USA). N-Methyl -N- *tert.*- butyldimethylsilyltrifluoroacetamide (MTBSTFA) with 1% *tert.*-butyldimethylsilyl chloride (*t*BDMS-Cl) and N-*tert.*-butyldimethylsilylimidazole (TBSIM) were either synthesized in this laboratory [17,18] or were purchased from Regis (Morton Grove, IL, USA) and United Chemical Technologies (Bristol, PA, USA), respectively. Toluene, hexane, dimethylformamide (DMF), tetrahydrofuran and pyridine were purchased from Aldrich and were redistilled prior to use. New Zealand orange roughy fish (*Hoplostethus atlanticus*) was purchased fresh from a local supermarket.

2.2. Gas chromatography

Direct capillary GLC analysis was performed with a Varian GLC, Model 3400CX, (Varian Associates, Park Ridge, IL, USA) equipped with dual flame ionization detectors (FID). The chromatographic column employed was a 30 m×0.25 mm I.D., fused-silica capillary column with bonded 0.25 μm film thickness of 100% methyl silicone (Quadrex Corporation, New Haven, CT, USA). The helium flow-rate was 5 ml/min, with injector and detector temperatures of 320°C and 340°C, respectively. After an initial hold of 4 min at 80°C, the column was temperature programmed at 5°C/min to 340°C. Alternatively, a column of identical dimensions, but possessing a 0.25 μm bonded film thickness of the intermediate polarity phase of methyl–50% phenyl silicone (Quadrex Corporation), was also employed and programmed from 80°C to 280°C at 4°C/min after an initial hold of 4 min. EZChrom Chromatography Data System (San Ramon, CA, USA) was employed for data acquisition and compression.

2.3. Gas chromatography–mass spectrometry

Mass spectra were obtained on a Kratos MS 25 mass spectrometer (Kratos, Manchester, UK) interfaced with a Carlo Erba Model 4160 gas chromatograph equipped with the 100% methyl silicone capillary column and programmed as indicated above. Mass spectra were recorded at 70 eV with an ionization current of 100 μA, a source temperature of 250°C, and a transfer temperature of 280°C. A Kratos Mach 3 Data System was employed for data acquisition, compression and fragment ion monitoring.

2.4. Alkyl alcohol standard solutions

Saturated primary alcohols, unsaturated primary alcohols, primary diols and secondary alcohols were prepared as individual groups to a final concentration of 1 mg each alcohol per ml of toluene. Solutions were kept in 25 ml serum vials sealed under an atmosphere of argon with a PTFE-faced silicone

septum. 1-Octadecanol was included as internal and reference standard in each standard fatty alcohol mixture. For derivatization reactions, 25–50 μl aliquots of these stock solutions were employed.

2.5. Isolation of fatty alcohols from animal tissue

Lyophilized New Zealand orange roughy fish (1.0 g) was extracted with chloroform–methanol–water (40:20:10, v/v). The chloroform layer was reduced to dryness in vacuo in a PTFE-faced screw-top test tube. The oily, waxy residue was then subjected to hydrolysis in 20 ml absolute methanol containing 4.0% sulfuric acid at 85°C for 2 h under argon atmosphere. After cooling the hydrolysis tube in an ice bath, 10 ml of ice cold water was then added and the contents mixed. The fatty alcohols and fatty acid methyl esters were then extracted with 10 ml hexane. Aliquots of the hexane extract were then subjected to thin-layer chromatography employing 5×20 cm plates coated with a 250 micron layer of silica gel G (Analtech, Newark, DE, USA), and using hexane–ethyl ether (1:1, v/v), as the developing solvent. Fatty alcohols (R_F=0.5–0.6), visualized with iodine vapor, were extracted from the silica with ethyl ether–methanol (10:1, v/v) and dried in vacuo.

2.6. Derivatization of fatty alcohols

To the respective Reactivial, equipped with a small PTFE-coated stir bar and a PTFE-faced silicone septum, 50 μl each of toluene, DMF and pyridine were added to the sample to be derivatized and then mixed. Then 100 μl of TBSIM and 200 μl of MTBSTFA, containing 1% *t*BDMS-Cl, were sequentially added with stirring. In some experiments, 300 μl of MTBSTFA, containing 1% *t*BDMS-Cl, was utilized without TBSIM. The Reactivial was then heated at 85°C for 30 min and allowed to cool to room temperature. Other reactions were performed at room temperature. Reactions were then monitored by GLC for completion. Derivatized alcohols were either directly analyzed or were washed prior to GLC analysis by adding 1.0 ml hexane to the reaction mixture which was then extracted three times each with 1.0 ml water to destroy unreacted TBSIM and MTBSTFA and to remove free imidazole, DMF and pyridine. The hexane solution could then be directly analyzed or reduced in volume via a jet of dry air. Verification of the bond locations of mono- and diunsaturated fatty alcohols was accomplished by the formation and GLC–MS analysis of their dimethyl disulfide adducts [19].

2.7. Statistics

Second order regression lines for carbon number versus retention data for primary alcohols, diols and secondary alcohols were generated by Sigma Plot Scientific Graphing Software, (Jandel Scientific Software, San Rafael, CA, USA), which employs least squares to fit data points (x_i, y_i) $i=1,\ldots, n$ to a polynomial of order p where $y=\beta_0+\beta_1 x+\beta_2 x^2+\ldots+\beta_p x^p$.

3. Results and discussion

3.1. Derivatization

tert.-Butyldimethylsilylation of all standard fatty alcohol mixtures with TBSIM and MTBSTFA containing 1% *t*BDMS-Cl was complete within 30 min at 85°C. Utilizing this derivatization cocktail and performing the derivatization at room temperature, it was observed that all primary and secondary fatty alcohols were completely derivatized within 30 min and 2–4 h, respectively. In the absence of TBSIM some secondary alcohols required longer derivatization times. Under these conditions, up to 2 h at 85°C or overnight at room temperature were required.

Substitution of ACN, DMSO, THF, ethyl acetate, chloroform or no solvent for toluene as the fatty alcohol solvent demonstrated no affect upon derivatization. Furthermore, it was observed that the *tert.*-butyldimethylsilylated fatty alcohols were very stable when stored in hexane or as a dried residue in a sealed Reactivial and showed no signs of degradation, even after 6 months. In contrast to TMS-compounds (8–10), *t*BDMS-derivatives are unique in their stability and ease of derivative purification from silylation reagents, by-products and solvents, and their subsequent ability to be concentrated prior to GLC–MS analysis makes their use ideal.

3.2. GLC separations

For illustration, a composite picture showing the separation of the *t*BDMS-derivatives of a primary alcohol and a diol standard mixture on a bonded 100% methyl silicone capillary GLC column and detected by FID is shown in Fig. 1A and B, respectively. As observed, each *t*BDMS-derivative presented a single sharp symmetrical chromatographic peak with no significant peak tailing or any indication of decomposition on the column. Notably, the ability to purify the *t*BDMS-derivatives prior to analysis, in contrast to TMS-derivatives, also permits fatty alcohols to be analyzed over a wide range of alkyl chain lengths. In this investigation, alkyl chain lengths of C_4 to C_{31} were studied, though chain lengths in excess of thirty-one carbons should readily be achievable. Separation of the standard *t*BDMS-fatty alcohols on a methyl–50% phenyl silicone fused-silica capillary column (not shown) provided the same general elution pattern as observed on the methyl silicone column (Fig. 1A,B) but with the unsaturated *t*BDMS-fatty alcohols eluting with, and after, the corresponding saturated *t*BDMS-fatty alcohol of corresponding chain length.

3.3. Retention data

Gas chromatographic retention data, expressed in methylene units for each *t*BDMS-ether of saturated and unsaturated primary alcohol, primary diol and secondary alcohol on a methyl silicone capillary column, are given in Table 1. As is generally observed on non-polar columns, for each subdivision of fatty alcohols, the *t*BDMS-ethers emerged from the methyl silicone capillary column primarily in the order of molecular mass and carbon chain length. Notably, branched fatty alcohols were baseline separated and eluted prior to the saturated *t*BDMS-ether of the respective straight-chain fatty alcohol of the

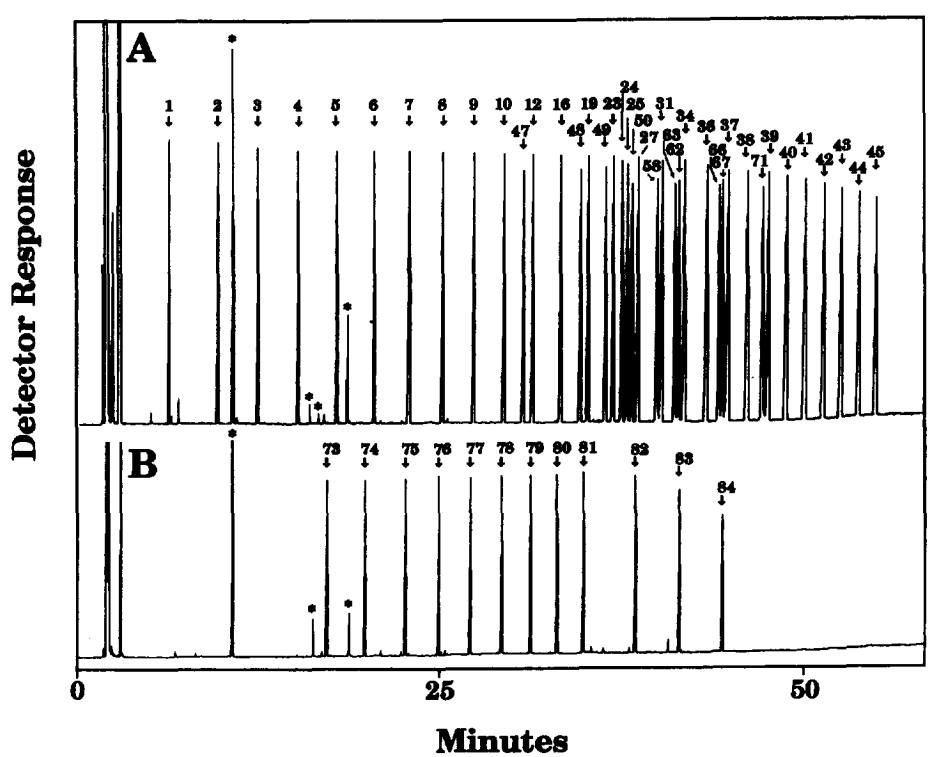

Fig. 1. GLC–FID chromatograms of *t*BDMS derivatives of standard mono- (A) and diols (B) performed on a 100% methyl silicone column, as described in Table 1. Single peaks were observed for each derivative and peak labels refer to compound numbers presented in Table 1. Peaks labeled with an asterisk (*) are related to the derivatization reagents.

Table 1
Methylene units (MU), relative weight ratios (RWR) and prominent fragment ions of fatty alcohols as their *tert.*-butyldimethylsilyl derivatives by GLC–MS analysis

Compound number	Alcohol	Trivial name (prefix)	Capillary column SPB-1 (bonded) MU (RWR)	Important/prominent mass spectral fragment ions (relative intensity)							
Primary alcohols – saturated				$M^{+\cdot}$	M−15	M−57					
1	1-Butanol		10.61 (1.08)	188 (7.4)	173 (11)	131 (100)	89 (69)	83 (13)	75 (92)	43 (44)	41 (30)
2	1-Pentanol	Valeryl	11.17 (1.08)	202 (1.1)	187 (8)	145 (100)	89 (65)	83 (10)	75 (98)	43 (35)	41 (25)
3	1-Hexanol	Caproyl	11.87 (1.08)	216 (0.6)	201 (6)	159 (100)	89 (68)	83 (36)	75 (97)	43 (29)	41 (25)
4	1-Heptanol	Heptyl	12.66 (1.07)	230 ([a])	215 (4)	173 (100)	89 (52)	83 (8)	75 (92)	43 (28)	41 (20)
5	1-Octanol	Caprylyl	13.48 (1.07)	244 ([a])	229 (4)	187 (100)	89 (61)	83 (9)	75 (96)	43 (41)	41 (31)
6	1-Nonanol	Pelargonyl	14.50 (1.05)	258 ([a])	243 (3)	201 (95)	89 (23)	83 (18)	75 (100)	43 (26)	41 (19)
7	1-Decanol	Capryl	14.99 (1.04)	272 ([a])	257 (3)	215 (100)	89 (55)	83 (40)	75 (94)	43 (38)	41 (27)
8	1-Undecanol		16.27 (1.04)	286 ([a])	271 (2)	229 (96)	89 (48)	83 (37)	75 (100)	43 (32)	41 (22)
9	1-Dodecanol	Lauryl	17.35 (1.04)	300 ([a])	285 (3)	243 (100)	89 (42)	83 (31)	75 (97)	43 (32)	41 (24)
10	1-Tridecanol		18.21 (1.03)	314 ([a])	299 (1)	257 (98)	89 (34)	83 (28)	75 (100)	43 (36)	41 (28)
11	12-Methyl-1-tetradecanol	(iso)	18.82 (0.98)	328 ([a])	313 (1)	271 (98)	89 (33)	83 (24)	75 (100)	43 (38)	41 (26)
12	1-Tetradecanol	Myristyl	19.11 (1.04)	328 ([a])	313 (2)	271 (96)	89 (38)	83 (31)	75 (100)	43 (37)	41 (30)
13	13-Methyl-1-pentadecanol	(iso)	19.60 (0.98)	342 ([a])	327 (1)	285 (93)	89 (33)	83 (23)	75 (100)	43 (40)	41 (28)
14	12-Methyl-1-pentadecanol	(anteiso)	19.70 (0.97)	342 ([a])	327 (1)	285 (94)	89 (21)	83 (29)	75 (100)	43 (25)	41 (32)
15	2-Methyl-1-tetradecanol		19.98 (1.02)	342 ([a])	327 (2)	285 (100)	129 (14)	117 (10)	75 (78)		
16	1-Pentadecanol		20.00 (1.01)	342 ([a])	327 (2)	285 (98)	89 (38)	83 (27)	75 (100)	43 (38)	41 (32)
17	14-Methyl-1-hexadecanol	(iso)	20.53 (0.98)	356 ([a])	341 (1)	299 (93)	89 (29)	83 (22)	75 (100)	43 (31)	41 (23)
18	13-Methyl-1-hexadecanol	(anteiso)	20.68 (0.98)	356 ([a])	341 (1)	299 (95)	89 (21)	83 (27)	75 (100)	43 (25)	41 (34)
19	1-Hexadecanol	Palmityl	21.07 (1.02)	356 ([a])	341 (1)	299 (96)	89 (39)	83 (32)	75 (100)	43 (43)	41 (34)
20	15-Methyl-1-heptadecanol	(iso)	21.48 (0.99)	370 ([a])	355 (1)	313 (92)	89 (29)	83 (20)	75 (100)	43 (42)	41 (27)
21	14-Methyl-1-heptadecanol	(anteiso)	21.55 (0.98)	370 ([a])	355 (1)	313 (94)	89 (17)	83 (24)	75 (100)	43 (26)	41 (31)
22	2-Methyl-1-hexadecanol		21.66 (1.01)	370 ([a])	355 (3)	313 (100)	129 (22)	117(15)	75 (95)		
23	1-Heptadecanol	Margaryl	21.73 (1.01)	370 ([a])	355 (1)	313 (96)	89 (38)	83 (26)	75 (100)	43 (39)	41 (27)
24	16-Methyl-1-octadecanol	(iso)	22.23 (0.98)	384 ([a])	369 (0.8)	327 (92)	89 (31)	83 (21)	75 (100)	43 (40)	41 (22)
25	15-Methyl-1-octadecanol	(anteiso)	22.50 (0.96)	384 ([a])	369 (0.7)	327 (93)	89 (20)	83 (29)	75 (100)	43 (27)	41 (31)
26	2-Methyl-1-heptadecanol		22.59 (1.01)	384 ([a])	369 (2)	327 (100)	129 (16)	117 (10)	75 (55)		
27	**1-Octadecanol**	**Stearyl**	**22.66 (1.00)**	**384 ([a])**	**369 (1)**	**327 (95)**	**89 (33)**	**83 (25)**	**75 (100)**	**43 (45)**	**41 (23)**
28	17-Methyl-1-nonadecanol	(iso)	22.71 (0.96)	398 ([a])	383 (0.5)	341 (91)	89 (32)	83 (23)	75 (100)	43 (48)	41 (29)
29	10-Methyl-1-octadecanol		23.30 (0.99)	398 ([a])	383 (2)	341 (100)	185 (7)	75 (97)			
30	2-Methyl-1-octadecanol		23.56 (0.99)	398 ([a])	383 (3)	341 (100)	129 (24)	117(18)	75 (98)		
31	1-Nonadecanol		23.63 (0.98)	398 ([a])	383 (1)	341 (94)	89 (40)	83 (31)	75 (100)	43 (49)	41 (33)
32	18-Methyl-1-eicosanol	(iso)	24.14 (0.95)	412 ([a])	397 (1)	355 (91)	89 (31)	83 (24)	75 (100)	43 (47)	41 (28)
33	2-Methyl-1-nonadecanol		24.31 (0.98)	412 ([a])	383 (4)	355 (100)	129 (28)	117(16)	75 (56)		
34	1-Eicosanol	Arachidyl	24.40 (0.97)	412 ([a])	397 (1)	355 (93)	89 (34)	83 (27)	75 (100)	43 (40)	41 (26)
35	2-Methyl-1-eicosanol		25.31 (0.98)	426 ([a])	411 (3)	369 (100)	129 (18)	117(16)	75 (100)		
36	1-Heneicosanol		25.45 (0.96)	426 ([a])	411 (0.8)	369 (93)	89 (29)	83 (24)	75 (100)	43 (49)	41 (30)
37	1-Docosanol	Behenyl	26.18 (0.96)	440 ([a])	425 (0.9)	383 (93)	89 (32)	83 (28)	75 (100)	43 (47)	41 (28)
38	1-Tricosanol		27.18 (0.95)	454 ([a])	439 (1)	397 (94)	89 (25)	83 (10)	75 (100)	43 (42)	41 (27)

Table 1. Continued

Compound number	Alcohol	Trivial name (prefix)	Capillary column SPB-1 (bonded) MU (RWR)	Important/prominent mass spectral fragment ions (relative intensity)								
39	1-Tetracosanol	Lignoceryl	28.10 (0.95)	468 ([a])	453 (0.6)	411 (92)	89 (26)	83 (7)	75 (100)	43 (48)	41 (27)	
40	1-Pentacosanol		29.22 (0.94)	482 ([a])	467 (0.9)	425 (91)	89 (24)	83 (8)	75 (100)	43 (43)	41 (24)	
41	1-Hexacosanol	Cerotyl	30.10 (0.92)	496 ([a])	481 (0.6)	439 (93)	89 (22)	83 (11)	75 (100)	43 (42)	41 (20)	
42	1-Heptacosanol		31.05 (0.91)	510 ([a])	495 (0.9)	453 (91)	89 (33)	83 (27)	75 (100)	43 (45)	41 (23)	
43	1-Octacosanol	Montanyl	32.04 (0.90)	524 ([a])	509 ([a])	467 (91)	89 (22)	83 (7)	75 (100)	43 (38)	41 (21)	
44	1-Nonacosanol		33.18 (0.90)	538 ([a])	523 ([a])	481 (92)	89 (33)	83 (27)	75 (100)	43 (45)	41 (23)	
45	1-Triacontanol	Melissyl	42.26 (0.89)	552 ([b])	537 ([a])	495 (93)	89 (18)	83 (8)	75 (100)	43 (38)	41 (22)	
46	1-Hentriacontanol		35.36 (0.87)	566 ([b])	551 (1)	509 (92)89 (33)	83 (27)	75 (100)	43 (45)	41 (23)		
Primary alcohols – unsaturated				$M^{+\cdot}$	M−15	M−57						
47	*cis*-9-Tetradecenol	Myristoleyl	18.84 (0.95)	326 ([b])	311 (1)	269 (49)	241 (8)	75 (100)	57 (7)	55 (25)	43 (11)	41(30)
48	*cis*-9-Hexadecenol	Palmitoleyl	20.67 (0.95)	354 ([b])	339 (1)	297 (44)	269 (5)	75 (100)	57 (8)	55 (24)	43 (12)	41(24)
49	*cis*-10-Heptadecenol		21.61 (0.94)	368 ([b])	353 (0.7)	311 (41)	283 (6)	75 (100)	57 (7)	55 (22)	43 (14)	41(21)
50	*cis*-9-Octadecenol	Oleyl	22.56 (0.94)	382 ([b])	367 (1)	325 (42)	297 (6)	75 (100)	57 (14)	55 (28)	43 (18)	41(30)
51	*cis*-11-Octadecenol	Vaccenyl	22.58 (0.92)	382 ([b])	367 (1)	325 (42)	297 (7)	75 (100)	57 (8)	55 (23)	43 (13)	41(19)
52	*cis*-12-Octadecenol		22.60 (0.93)	382 ([b])	367 (0.8)	325 (44)	297 (6)	75 (100)	57 (7)	55 (23)	43 (7)	41(19)
53	9*c*,12*c*-Octadecadienol	Linoleyl	22.48 (0.89)	380 ([b])	365 (3)	323 (34)	295 (7)	75 (100)	57 (8)	55 (20)	43 (7)	41(22)
54	9*c*,12*c*,15*c*-Octadecatrienol	Linolenyl	22.52 (0.84)	378 ([b])	363 (1)	321 (28)	293 (6)	75 (100)	57 (4)	55 (15)	43 (5)	41(18)
55	*trans*-6-Octadecenol		22.49 (0.91)	382 ([b])	367 (1)	325 (56)	297 (4)	75 (100)	57 (13)	55 (19)	43 (16)	41(18)
56	*cis*-7-Octadecenol		22.49 (0.93)	382 ([b])	367 (0.9)	325 (42)	297 (3)	75 (100)	57 (16)	55 (25)	43 (21)	41(27)
57	*cis*-13-Octadecenol		22.61 (0.93)	382 ([b])	367 (0.7)	325 (47)	297 (5)	75 (100)	57 (4)	55 (23)	43 (7)	41(15)
58	*cis*-10-Nonadecenol		23.30 (0.92)	396 ([b])	381 (0.6)	339 (43)	311 (6)	75 (100)	57 (9)	55 (23)	43 (13)	41(18)
59	*cis*-5-Eicosenol		24.25 (0.91)	410 ([b])	395 (0.6)	353 (44)	325 (5)	75 (100)	57 (18)	55 (22)	43 (33)	41(28)
60	*cis*-8-Eicosenol		24.21 (0.90)	410 ([b])	395 (0.5)	353 (42)	325 (6)	75 (100)	57 (16)	55 (24)	43 (10)	41(21)
61	*cis*-11-Eicosenol	Gondyl	24.21 (0.90)	410 ([b])	395 (0.5)	353 (48)	325 (5)	75 (100)	57 (12)	55 (27)	43 (16)	41(23)
62	*cis*-13-Eicosenol		24.31 (0.90)	410 ([b])	395 (0.5)	353 (42)	325 (6)	75 (100)	57 (8)	55 (28)	43 (16)	41(22)
63	11*c*,14*c*-Eicosadienol		24.20 (0.86)	408 ([b])	393 (0.6)	351 (32)	323 (5)	75 (100)	57 (5)	55 (20)	43 (6)	41(18)
64	*cis*-11-Heneicosenol		25.09 (0.89)	424 ([b])	409 (0.6)	367 (45)	339 (6)	75 (100)	57 (15)	55 (23)	43 (18)	41(27)
65	*cis*-13-Heneicosenol		25.27 (0.89)	424 ([b])	409 (0.6)	367 (46)	339 (5)	75 (100)	57 (13)	55 (21)	43 (15)	41(22)
66	*cis*-11-Docosenol	Cetolyl	25.93 (0.88)	438 ([b])	423 (0.7)	381 (47)	353 (6)	75 (100)	57 (13)	55 (28)	43 (16)	41(25)
67	*cis*-13-Docosenol	Erucyl	26.03 (0.87)	438 ([b])	423 (0.6)	381 (43)	353 (5)	75 (100)	57 (12)	55 (27)	43 (15)	41(23)
68	13*c*,16*c*-Docosadienol		26.00 (0.82)	436 ([b])	421 (0.5)	379 (43)	351 (4)	75 (100)	57 (6)	55 (22)	43 (8)	41(20)
69	*cis*-14-Triacosenol		26.92 (0.85)	452 ([b])	437 (0.8)	395 (48)	367 (7)	75 (100)	57 (11)	55 (26)	43 (15)	41(23)
70	*cis*-15-Triacosenol		27.11 (0.85)	452 ([b])	437 (0.7)	395 (37)	367 (7)	75 (100)	57 (13)	55 (21)	43 (15)	41(23)
71	*cis*-13-Tetracosenol		27.80 (0.84)	466 ([b])	451 (0.8)	409 (35)	381 (7)	75 (100)	57 (16)	55 (30)	43 (17)	41(25)
72	*cis*-15-Tetracosenol	Nervonyl	27.89 (0.83)	466 ([b])	451 (0.8)	409 (33)	381 (8)	75 (100)	57 (15)	55 (29)	43 (16)	41(22)
Primary diols				$M^{+\cdot}$	M−15	M−57						
73	1,2-Ethanediol		13.32 (1.43)	290 ([b])	275 (7)	233 (82)	189 (17)	147 (100)	133 (26)	117 (32)	59 (38)	41 (30)
74	1,3-Propanediol		14.15 (1.40)	304 ([b])	289 (5)	247 (47)	189 (18)	147 (100)	133 (25)	117 (27)	59 (24)	41 (33)
75	1,4-Butanediol		15.39 (1.36)	318 ([b])	303 (0.5)	261 (10)	189 (37)	147 (100)	133 (21)	73 (32)	55 (14)	41 (9)

76	1,5-Pentanediol	16.27 (1.33)	332 ([b])	317 ([a])	275 (82)	189 (28)	147 (100)	133 (18)	109 (11)	69 (73)	41 (18)
77	1,6-Hexanediol	17.31 (1.31)	346 ([b])	331 ([a])	289 (5)	189 (25)	147 (100)	133 (15)	83 (88)	55 (89)	41 (30)
78	1,7-Heptanediol	18.20 (1.29)	360 ([b])	345 ([b])	303 (5)	189 (34)	147 (100)	133 (17)	97 (66)	75 (60)	55 (91)
79	1,8-Octanediol	19.12 (1.27)	374 ([b])	359 ([b])	317 (4)	189 (33)	147 (84)	133 (19)	75 (87)	69 (100)	41 (49)
80	1,9-Nonanediol	20.01 (1.24)	388 ([b])	373 ([b])	331 (3)	189 (27)	147 (44)	133 (10)	83 (54)	69 (100)	55 (40)
81	1,10-Decanediol	20.98 (1.22)	402 ([b])	387 ([b])	345 (3)	189 (26)	147 (67)	133 (13)	97 (50)	83 (100)	75 (91)
82	1,12-Dodecanediol	22.65 (1.21)	430 ([b])	415 ([b])	373 (3)	189 (23)	147 (51)	133 (10)	97 (52)	83 (65)	75 (100)
83	1,14-Tetradecanediol	24.39 (1.19)	458 ([b])	443 ([b])	401 (3)	189 (32)	147 (53)	133 (11)	97 (43)	83 (53)	75 (100)
84	1,16-Hexadecanediol	26.18 (1.15)	486 ([b])	471 ([b])	429 (2)	189 (23)	147 (51)	133 (10)	97 (52)	83 (63)	75 (100)
Secondary alcohols			$M^{+\cdot}$	M−15	M−57						
85	2-Hexanol	10.98 (1.02)	216 ([a])	201 (5)	159 (92)	75 (100)					
86	2-Heptanol	11.92 (1.02)	230 ([a])	215 (4)	173 (93)		159 (17)	75 (100)			
87	2-Octanol	12.85 (1.01)	244 ([a])	229 (4)	187 (93)		159 (17)	75 (100)			
88	2-Nonanol	13.82 (1.01)	258 ([a])	243 (4)	201 (91)		159 (16)	75 (100)			
89	5-Decanol	14.40 (0.94)	272 ([b])	257 (2)	215 (72)		201 (20)	75 (100)			
90	4-Decanol	14.44 (0.95)	272 ([a])	257 (2)	215 (48)	229 (5)	187 (12)	75 (100)			
91	2-Decanol	14.80 (1.03)	272 ([a])	257 (4)	215 (93)		159 (16)	75 (100)			
92	2-Undecanol	15.75 (0.97)	286 ([b])	271 (3)	229 (93)		159 (18)	75 (100)			
93	2-Dodecanol	16.65 (0.97)	300 ([b])	285 (4)	243 (92)		159 (19)	75 (100)			
94	2-Tetradecanol	18.59 (0.97)	328 ([b])	313 (2)	271 (92)		159 (18)	75 (100)			
95	2-Pentadecanol	19.31 (0.97)	342 ([b])	327 (2)	285 (94)		159 (12)	75 (100)			
96	5-Hexadecanol	19.71 (0.93)	356 ([b])	341 (3)	299 (73)		201 (17)	75 (100)			
97	3-Hexadecanol	20.17 (0.93)	356 ([b])	341 (1)	299 (63)	327 (8)	173 (18)	75 (100)			
98	2-Hexadecanol	20.44 (0.96)	356 ([b])	341 (3)	299 (91)		159 (20)	75 (100)			
99	4-Heptadecanol	20.74 (0.91)	370 ([b])	355 (1)	313 (43)	327 (7)	187 (12)	75 (100)			
100	3-Heptadecanol	21.15 (0.91)	370 ([b])	355 (1)	313 (38)	341 (4)	173 (19)	75 (100)			

The *t*BDMS derivatives of the fatty alcohols were analyzed by GLC–MS employing a 30 m×0.25 mm I.D. capillary column possessing a 0.25 μm film of bonded 100% methyl silicone phase (SPB-1). The helium flow-rate was 5 ml/min, with injector and detector temperatures of 320°C and 340°C, respectively. After an initial hold of 4 min at 80°C the column was temperature programmed at a rate of 5°C/min to 340°C. Chromatographic retention data are expressed in methylene units (MU) and all relative weight responses (RWR) are expressed relative to 1-octadecanol. Mass spectra were recorded at 70 eV with an ionization current of 100 μA, a source temperature of 250°C, and a transfer temperature 280°C.

[a] Observed but below 0.5% relative intensity.

[b] Not observed.

same carbon number and that the order of their elution is structurally specific [20]. As also observed for branched fatty acid methyl esters [20], the *t*BDMS derivatives of iso-fatty alcohols eluted before their respective anteiso-form. Similarly, the *t*BDMS-ethers of unsaturated fatty alcohols eluted before their respective saturated analog. Though unsaturated, branched fatty alcohols were not available for this study, it is speculated that they would elute prior to their saturated analog.

Second order regression curves (not shown), generated from the plots of the carbon number versus retention time data from the methyl silicone capillary column for primary and C-2 secondary alcohols and for diols, demonstrated highly significant correlations (r^2=0.9996 for each). Each type of *t*BDMS-alkyl ether produced a unique curve permitting predictability for each class of alcohol, as well as its carbon number.

3.4. Quantitative aspects

The relative weight response (RWR) of the *t*BDMS-ether of each fatty alcohol is also presented in Table 1. The relative standard deviation (R.S.D.) for each RWR on the methyl silicone capillary column was less than 1.46% in all cases demonstrating the excellent precision in the RWR of each *t*BDMS-ether derivative.

Employing fatty alcohol standards, a broad linear response curve in the range of 0.27 to 150 nmol was obtained for each *t*BDMS-ether of saturated and unsaturated primary alcohol, primary diol and secondary alcohol using FID as the GLC detector. By utilizing GLC–MS, the combination of retention data, unique and structurally informative fragment ions (discussed in Section 3.5) and system sensitivity, a ten-fold increase in detection is achieved over FID.

3.5. Mass spectrometry

The *t*BDMS-ether of each saturated and unsaturated primary alcohol, primary diol and secondary fatty alcohol was subjected to combined GLC–MS analysis and the results showing both important and prominent fragment ions are presented in Table 1. Similar to TMS-derivatives [21], most *t*BDMS derivatized alcohols, molecular ions $[M^{+\cdot}]$ and $[M-15]^+$ fragment ions were very weak or not observed. With the exception of derivatized diols, discussed later, the *t*BDMS-ethers of fatty alcohols, in contrast to TMS-derivatives, produced mass spectra in which each possessed a dominant (base fragment ion or >90% of base fragment ion) or a prominent $[M-57]^+$ fragment ion, i.e., $R\text{-}O^+=Si(CH_3)_2$, in the high molecular mass mass spectral region and from which mass of the molecule and degree of unsaturation could be deduced. For all saturated primary and secondary *t*BDMS-ethers, the molecular mass difference between $[M-57]^+$ and *m/z* 75 divided by 14 (i.e., a methylene unit, CH_2) yields the integer number of carbons in R. For mono-, di- and tri-unsaturated fatty alcohols the same manipulation will produce a decimal of 0.14, 0.29 and 0.43, respectively, lower than the whole integer number of carbons in the molecule. Continued fragmentation of the $R\text{-}O^+=Si(CH_3)_2$ through hydrogen rearrangement and adjacent σ-bond dissociation of the respective alkyl chain (R) from the oxygen heteroatom, i.e., $[M-\cdot C(CH_3)_3-C_nH_{2n}]^+$, results in the formation of $HO^+=Si(CH_3)_2$, *m/z* 75. Notably, this fragment ion is commonly observed to serve as the base fragment or prominent fragment ion in most mass spectra.

In addition to the above fragment ions, other important fragment ions indicative of each type of fatty alcohol, and their relative intensities, are also given in Table 1. For *t*BDMS derivatives of unbranched saturated and primary alcohols, the relative intensities of *m/z* 89 and *m/z* 43 are characteristically greater than *m/z* 83 and *m/z* 41, respectively. While the latter is also observed for the derivatized iso-fatty alcohols, the mass spectra of the *t*BDMS-ethers of anteiso-fatty alcohols contrast in that they display higher *m/z* 83 and *m/z* 41 relative intensities over *m/z* 89 and *m/z* 43, respectively. Though not presented in Table 1, the typical fragment ion series of $[C_nH_{2n+1}]^+$ (i.e., *m/z* 29, 43, 57, 71, 85, 99), which result from σ-bond dissociation within the saturated alkyl side-chains, are also observed and are contributed to by the fragment ions of *m/z* 99, 57, 41 and 29 which result from fragmentation of the *t*BDMS or $-C(CH_3)_3$ group.

In addition to the iso- and anteiso-fatty alcohols, noted above, the mass spectra of *t*BDMS-ethers of fatty alcohols with methyl branches at C-2 and C-10

present with $[M-57]^+$ base fragment ions and fragment ions *m/z* 117 $[(CH_3)CH_2CH_2\text{-}O^+=Si(CH_3)_2]$ and *m/z* 185 $[(CH_2=CH)(CH_2)_6\text{-}O^+=Si(CH_3)_2]$, respectively, indicating branch locations along the alkyl chain.

The other prominent fragment ions of the *t*BDMS-ethers of unsaturated primary alcohols include the hallmark fragment ion $[M-57-28]^+$, indicative of unsaturation, and the fragment ions *m/z* 55 and *m/z* 41 whose relative intensities are greater than *m/z* 57 and *m/z* 43, respectively. Notably, the $[M-57]^+$ fragment ionS of derivatized unsaturated fatty alcohols are 2, 4, 6, etc., amu less than the corresponding saturated alcohol indicating 1, 2, 3, etc., degrees of unsaturation, respectively. Due to the normally strong tendency for alkenes to isomerize via migration of double bonds during electron impact ionization [22], no information regarding bond location is obtained from the fragmentation pattern of the *t*BDMS-ethers of unsaturated fatty alcohols. In cases where two or more monoenes or dienes are present and not resolvable, bond locations can readily be verified via GLC–MS analysis of their respective dimethyl disulfide adducts [19].

The mass spectra of $(t\text{BDMS})_2$-ethers of primary diols displayed no molecular ions $[M^{+\cdot}]$, weak or no $[M-15]^+$ fragment ions, and a prominent or weak fragment ion at $[M-57]^+$ for derivatized diols of low and high molecular mass, respectively. All other major fragment ions relate to the fragmentation or rearrangement of the *t*BDMS function. The base fragment ion is typically *m/z* 147 or *m/z* 75 for short and long chain derivatized diols, respectively.

Derivatized secondary alcohols present mass spectra with prominent $[M-57]^+$ fragment ions and fragment ion *m/z* 75, which serves as the base fragment ion. All *t*BDMS-ethers of 2-hydroxy fatty alcohols displayed a *m/z* 159 $[CH_3CH=O^+\text{-}Si(CH_3)_2(C(CH_3)_3]$ fragment ion which results from the cleavage between C-2 and C-3, with loss of the largest alkyl chain, indicating C-2 as the site where the ether resides. For the *t*BDMS-ethers of 3-, 4- and 5-hydroxy fatty alcohols, fragment ions representing cleavage and charge retention at the ether possessing

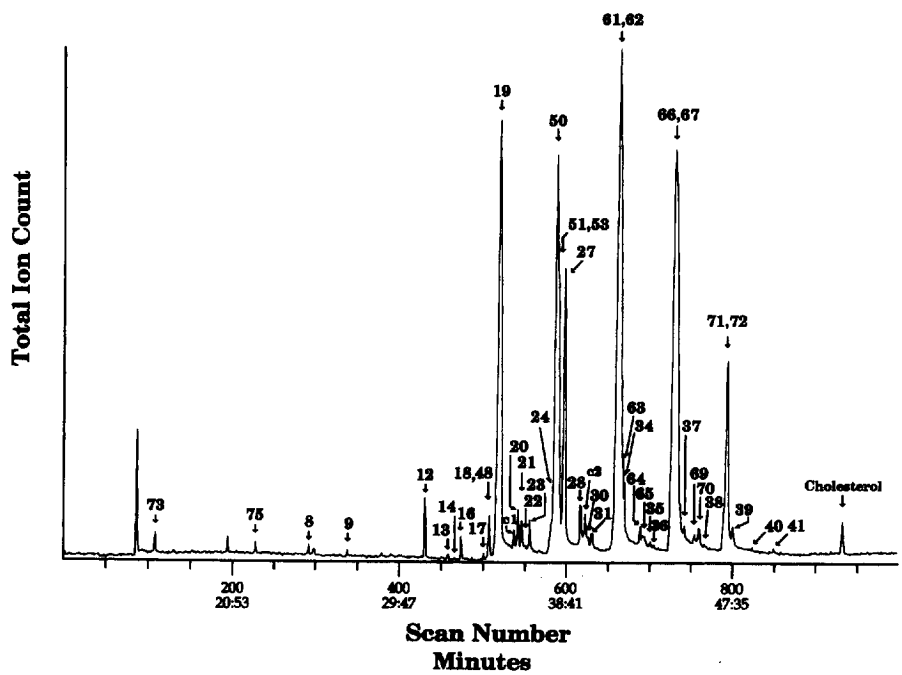

Fig. 2. Total ion mass spectrogram of the *t*BDMS-ethers of the fatty alcohols isolated from New Zealand orange roughy fish analyzed by GLC–MS separated on a 100% methyl silicone column, as described in Table 1. Peak numbers refer to numbered compound presented in Tables 1 and 2. Peaks designated with a letter label (i.e., ^{c1}), which are not in the standard mixtures presented in Table 1, are identified in Table 2.

Table 2
Composition of fatty alcohols in orange roughy fish

No.	Fatty alcohol	% of Total (reported[a])
8	1-Undecanol	0.06[b]
9	1-Dodecanol	0.05[b]
10	1-Tridecanol	0.03 (0.03)
12	1-Tetradecanol	1.78 (2.08)
13	1-Pentadecanol (iso)	0.14 (0.16)
14	1-Pentadecanol (anteiso)	0.01[b]
16	1-Pentadecanol	0.63 (0.54)
17	1-Hexadecanol (iso)	0.17 (0.24)
18	1-Hexadecanol (anteiso)	0.01[c] (0.02)
19	1-Hexadecanol	20.22 (22.16)
20	1-Heptadecanol (iso)	0.45 (0.58)
21	1-Heptadecanol (anteiso)	0.33 (0.32)
22	2-Methyl-1-hexadecanol	0.09[b]
23	1-Heptadecanol	0.31 (0.49)
24	1-Octadecanol (iso)	0.88 (1.05)
27	**1-Octadecanol**	8.16 (5.95)
28	1-Nonadecanol (iso)	0.38 (0.04)
30	2-Methyl-1-octadecanol	0.10[b]
31	1-Nonadecanol	0.22 (0.17)
34	1-Eicosanol	0.39 (0.52)
35	2-Methyl-1-eicosanol	0.06[b]
36	1-Heneicosanol	0.07 (0.06)
37	1-Docosanol	0.15 (0.12)
38	1-Tricosanol	0.09[b]
39	1-Tetracosanol	0.14[b]
40	1-Pentacosanol	<0.01[b]
41	1-Hexacosanol	<0.01[b]
48	*cis*-9-Hexadecenol	0.54 (0.78)
	cis-9-Heptadecenol	0.11 (0.30)[c1]
50	*cis*-9-Octadecenol	12.15 (11.35)
51	*cis*-11-Octadecenol	2.11 (2.76)
53	9*c*,12*c*-Octadecadienol	0.15 (0.12)
	cis-11-Nonadecenol	0.22 (0.33)[c2]
61	*cis*-11-Eicosenol	16.83 (16.17)
62	*cis*-13-Eicosenol	8.46 (9.10)
63	11*c*,14*c*-Eicosenol	0.16 (0.28)
64	*cis*-11-Heneicosenol	0.16[b]
65	*cis*-13-Heneicosenol	0.12[b]
66	*cis*-11-Docosenol	13.61 (12.98)
67	*cis*-13-Docosenol	3.22 (3.80)
69	*cis*-14-Tricosenol	0.09 (0.11)
70	*cis*-15-Tricosenol	0.18[b]
71	*cis*-13-Tetracosenol	1.88 (0.51)
72	*cis*-15-Tetracosenol	3.94 (2.97)
73	1,2-Ethanediol	0.87[b]
75	1,4-Butanediol	0.26[b]

[a] See Ref. [7].
[b] Not previously reported.
[c] Not in standard mixture.

carbon, for both the short and long alkyl groups, are observed and confirm ether locations.

3.6. Analysis of extracted fatty alcohols

One of the most complex mixtures of fatty alcohols found in nature is present in the fat extracted from orange roughy fish [7]. Fig. 2 presents the GLC–MS chromatogram of the *t*BDMS derivatives of a roughy fish fatty alcohol preparation. By utilizing both retention data and $[M-57]^+$ fragment ions, and other key fragment ions, forty-one fatty alcohols were identified and included many saturated and unsaturated, and iso- and anteiso-fatty alcohols, as well as two diols, 1,2-ethanediol and 1,4-butanediol (Table 2). Via this procedure, fourteen fatty alcohols, not previously reported in orange roughy, were observed (Table 2).

In conclusion, a sensitive method is described in which saturated and unsaturated primary alcohols, iso- and anteiso-primary alcohols, secondary alcohols and primary diols are derivatized to their respective *t*BDMS-ether derivatives and analyzed by GLC and GLC–MS. The derivatives are readily prepared, stable, have excellent capillary column characteristics and can be purified from silylation reagents and solvents making this procedure readily amenable to small samples. In addition, the *t*BDMS-ether of each alcohol displayed a prominent $[M-57]^+$ fragment ion, which when combined with retention data and other informative fragment ions, allows for fatty alcohol identification.

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